STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL. XVI. DOUBLE EGGS.[1]

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Among the eggs of the domestic fowl an egg which contains another egg is quite rare, but one or more such specimens have been observed by most persons who have handled large numbers of eggs. This phenomenon has excited the interest of poultrymen and scientists and a number of specimens have been described in the agricultural and scientific literature. The purpose of the present paper is to describe several specimens observed at the Maine Agricultural Experiment Station which have been laid or have been found partly formed within the oviduct at autopsy and to discuss the formation of these abnormalities from the physiological point of view.

Parker (12) published an extensive bibliography on the subject and discussed at some length the recorded cases in connection with four cases which he had himself observed. Von Durski (6) also cites a number of other cases collected from the literature and gives a bibliography. Summarizing these cases briefly we arrive at the following conclusions:

1. Either a normal egg or a dwarf egg which contains little or no yolk may be enclosed with a normal yolk in a second set of normal egg envelopes. The included egg may lie near the yolk of the including egg or it may be enclosed only in the outer layers of albumen.

2. Either a normal or a dwarf egg may be enclosed in a set of normal egg envelopes without any yolk being present in the enclosing egg.

3. When the included egg has a blunt and a pointed end which are distinguishable, it always lies with its pointed end toward the pointed end of the including egg.

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 97.

4. When a yolk is present in the including egg it always lies toward the blunt end, while the included egg occupies the pointed end.

These facts indicate that a normal or a dwarf egg which has passed through the duct as far as the isthmus (when the included egg is only membrane covered) or shell gland (when it has a hard shell) may be returned up the duct without reversing the poles of its long axis. Somewhere, usually in the albumen region,[1] the direction may again be reversed. If the succeeding egg is already forming in the duct the egg which has been forced back lies in the duct posterior to this. As the forming egg completes itself enclosing its predecessor the enclosed egg will of necessity lie in the pointed end and the extent that it is imbedded within the albumen of the enclosing egg will depend on the level of the duct where the two eggs unite. If there is no second egg in the duct the egg which has been returned may stimulate the duct to the secretion of the egg envelopes.

The sixteen double, or enclosed eggs which we have had the opportunity of examining include specimens which show many interesting peculiarities. They will, therefore, be described individually. They may, however, be classified according to their general structure into (1) double eggs with the enclosing egg a normal egg and (*a*) the enclosed egg also normal, or (*b*) the enclosed egg a dwarf egg; and (2) double eggs in which the enclosing egg does not contain a yolk but is simply a set of egg envelopes enclosing (*a*) a normal egg, or (*b*) a dwarf egg.

I. Double Eggs in Which the Enclosing Egg is a Normal Egg.

This group includes specimens 1 to 5.

(*a*) *The Enclosed Egg is Also a Normal Egg in Specimens 1 and 2.*

Specimen 1 (Plate I) was produced at the Maine Station poultry plant. The external appearance of this egg was that of a large membranous sac distinctly pointed. At the blunt end

[1] Gruvel (7) describes a case where the included egg lies between the egg membranes of the including egg indicating that the returned egg came upon its successor in the isthmus instead of within the albumen region, as in all the other cases known to the author.

the sac was continued into a stalk about the size of the index finger. The sac and appendage were covered with a very thin layer of shell. The membrane was ruptured at the foot of the stalk, exposing a large area of the shell of the normal egg which was included. The torn edges of membrane were stuck tight to the shell of the included egg apparently by the thin layer of shell which covered the membrane. There were also folds in the membrane at the foot of the stalk and the inner parts of these folds were not covered with shell. Apparently this rupture and folding of the membrane took place before the shell on the enclosing egg was formed. It will be noted that the enclosed egg lies entirely to one side with its pointed end toward the pointed end of the enclosing egg. At the pointed end of the enclosing egg there is a fresh rupture. From this albumen and yolk were protruding when the egg was found. The yolk had been broken but appeared to have been a normal yolk. The stalk was still filled with albumen. Evidently this rupture had occurred when the egg was laid and was no doubt due to the large size of the egg. The enclosed egg was normal in all respects. Apparently this normal egg had been forced back up the oviduct without reversing its polarity. It apparently met the succeeding egg at the posterior end of the albumen secreting region since it evidently lay quite outside the albumen of this egg. The shape of the enclosing egg indicates that the two eggs passed through the isthmus side by side. There was no membrane around the enclosed egg. That is it did not receive a membrane when it passed up the duct.

Specimen 2 (Plate II. and Figs. 1 and 2) was presented to the Station by Mrs. Ethel Pike, of Winthrop, Maine. Several days elapsed after the egg was laid before it reached the Station laboratory. The egg was well protected but some evaporation had evidently occurred as there were folds in the enclosing membrane which was without shell. Within this membrane lay two normal eggs with their long axes parallel. One of these was a normal egg enclosed in an egg membrane and shell. The other was a normal yolk enclosed in a normal albumen envelope. There was a shallow layer of thin albumen common to the two eggs. It will be seen in the photograph that the folds in the

enclosing membrane were all in the part which covered the naked egg as the shell of the included egg maintained the shape of that part of the outer membrane which covered it. As in the case of specimen number 1, the normal egg was evidently returned up the duct meeting the succeeding egg in the lower part of the albumen secreting region. The two then passed back through the isthmus, with their long axes parallel to each other. Whether or not they were also parallel to the long axis of the duct is impossible to tell since the complete egg was not pointed. In this case also there was no membrane surrounding the shell of the enclosed egg. That is, it did not receive a membrane as it passed up through the isthmus.

(*b*) *The Enclosed Egg was a Dwarf Egg in Specimens 3, 4, and 5.*

Specimen 3 was produced at the Maine Station poultry plant. It had the external appearance of a double-yolked egg with normal shell membranes and shell. The dimensions of this egg were: length 72.1 mm., breadth 46.0 mm., and weight 82.0 gm. On opening the egg it was found to contain at its blunt end a normal yolk which weighed 18.06 gm. and at its pointed end a small spherical soft-shelled dwarf egg which weighed 13.07 gm. A photograph of the contents of this egg is shown in Plate III.,

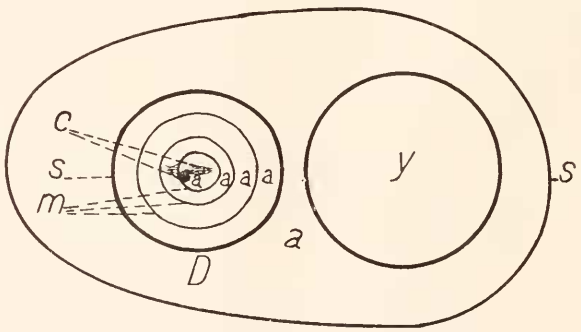


FIG. 1. Diagrammatic sketch showing the structure of double egg No. 3. *D* = dwarf egg composed of four concentric dwarf eggs; *Y* = normal yolk; *a* = albumen; *c* = chalazal-like fibers; *m* = egg membrane; *s* = shell.

Fig. 1, and a diagrammatic sketch of its structure is shown in Text-figure 1.

The dwarf egg lay in the pointed end and the yolk in the

blunt end of the enclosing egg. The fact that the dwarf egg is enclosed in only the outer thick albumen layers may readily be seen from the photograph. No chalazæ were visible in the enclosing egg. The structure of the dwarf egg was quite complex. It consisted of a series of four concentric egg membranes separated from each other by layers of clear thick albumen. Within the inner membrane was a mass of chalazal-like coagulation fibers surrounded by thick albumen. Attached to one end of the innermost egg was a mass of coagulation fibers. There was no shell on any of the egg membranes except the outer one. The structure of this egg indicates that a very small dwarf egg passed back from the isthmus to the albumen-secreting region, acquiring some chalazal fibers and a small amount of albumen. It then passed into the isthmus, received another membrane, and was then returned to the albumen-secreting region, where it received another albumen layer. Passing again to the isthmus it received its third membrane. It was again returned to the albumen region where it received another layer of albumen. It then passed through the isthmus into the shell gland receiving an egg membrane and a thin layer of shell. It was then returned again to the lower portion of the albumen-secreting region where it met a normal yolk surrounded by several layers of albumen and became enclosed with this in a few layers of albumen and egg membrane and shell. The fact that the albumen separating the concentric egg membranes of the enclosed dwarf egg was in each case the clear thick albumen, secreted so far as is known only in the albumen secreting region, compels the conclusion that the egg passed from isthmus to albumen-secreting region several times. This indicates a considerable disturbance of the normal movements. Whatever the nature of this disturbance the egg record of the bird shows that it was of temporary character, since the bird had been producing and continued to produce normal eggs in regular series. The double egg was the first egg of a two egg clutch. It followed a four-day non-production period, on the last day of which the bird nested but did not lay.

Specimen 4 was brought to the Maine Station biological laboratory by Dr. O. A. Johannsen. The egg had been broken

for domestic use, so that its internal arrangement could not be certainly ascertained. It was of a practically normal size and contained a normal yolk and a very small dwarf egg which weighed 4 gm. This dwarf egg contained a small piece of hardened secretion about the size of a pinhead surrounded by layers of albumen which were distinctly visible by transmitted light. The membrane of the dwarf egg was quite thick and the shell very thin. After a short stay in the shell gland the dwarf egg had apparently been returned to the albumen-secreting region without receiving a membrane on its upward passage. Here it met and became enclosed in the succeeding egg.

Specimen 5 was brought to this laboratory by Mr. H. W. Smith. This egg had been broken for laboratory purposes. He said that the egg was of normal external appearance and average size. He did not note the relation of the internal structures to the poles of the egg. The egg contained a normal yolk. Separated from this by a few layers of thick albumen was a worm-like membrane-covered dwarf egg. This dwarf egg

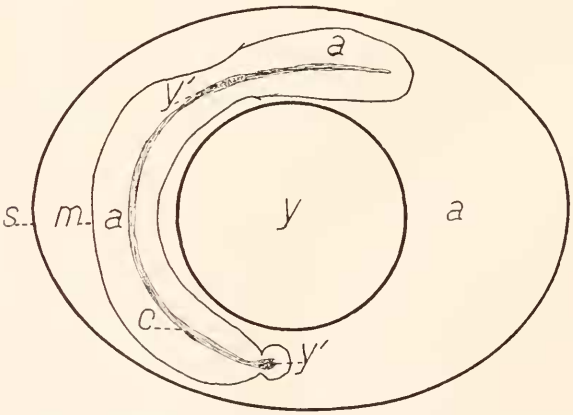


FIG. 2. Diagram showing structure of dwarf eggs No. 5. a = albumen; c = chalazal-like fibers, m = egg membrane; s = shell; y = normal yolk; y' = yolk droplets.

was bent around the yolk. The yolk and dwarf egg were included in a common albumen envelope. The structure of this egg is shown in Fig. 2. The resemblance of the dwarf egg to a simple organism of some kind was striking. Running through

the middle was a string of coagulation fibers like untwisted chalazal threads. Mixed with these at certain points were small droplets of yolk. The membrane covering the dwarf egg was complete at one end and open at the other. The albumen surrounding the chalazal-like core was thick. The chalazal core surrounded by thick albumen continued beyond the membrane at the open end. This naked portion separated definitely from the surrounding albumen when the dwarf egg was taken from the albumen. Evidently this cylindrical dwarf egg had passed partly into the isthmus and had then been returned into the albumen region where it became enclosed in the succeeding egg.

II. Double Eggs in Which the Enclosing Egg is a Set of Egg Envelopes with no Apparent Nucleus Except the Enclosed Egg.

(*a*) *The Enclosed Egg was a Normal Egg or at Least Had a Normal Yolk in Specimens 6, 7, 8, 9 and 10.*

Specimen 6 was laid by a normal bird belonging to the Maine Station flock. In external appearance it resembled a large hard-

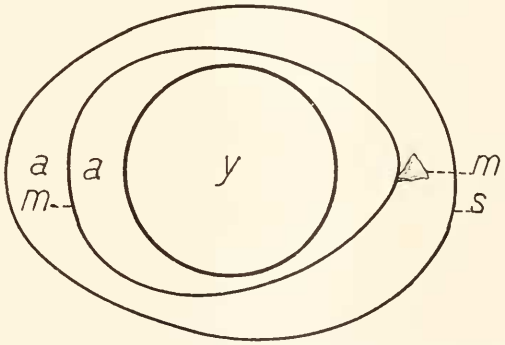


Fig. 3. Diagram showing the structure of double egg No. 6. *a* = albumen; *m* = membrane; *s* = shell, *y* = normal yolk.

shelled double-yolked egg. A diagram showing the structure of this egg is shown in Fig. 3. The entire egg weighed 94.74 gm. When the egg was opened it was found to contain a membrane-covered egg surrounded by a layer of thick and a layer of thin albumen. This albumen weighed 57.39 gm. The weight of

the shell was 8 gm. The enclosed egg weighed 29.36 gm. Attached to one pole of the enclosed egg by the inner layer of thick albumen was a white mass which on close examination appeared to be a triangular piece of egg membrane. The enclosed egg was membrane-covered. Its contents resembled a large normal egg as it appears while it is in the upper part of the albumen-secreting region of the oviduct. That is, it consisted of a large yolk (weight 23.16 gm.) surrounded by a thin layer of very thick clear albumen which adhered closely to the yolk. This albumen weighed only 5.57 gm. The enclosed egg was distinctly pointed and lay with its pointed end toward the blunt end of the enclosing egg. It has been noted that in all the cases described and reviewed by Parker (12) when a pointed end was distinguishable in both included and including eggs the pointed end of the former always lay toward the pointed end of the latter. In this particular, therefore, the above described case differs from those known to Parker. Since specimen No. 7

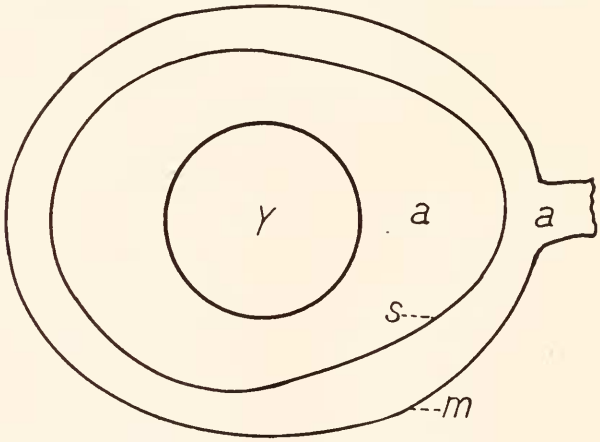


FIG. 4. Diagram showing the structure of double egg No. 7. a = albumen; m = egg membrane; s = shell; y = normal yolk.

also shows the poles of the included egg in the reverse direction to those of the including egg, this case will be described and the reversal of axes will then be discussed.

Specimen No. 7 was brought to this laboratory by Dr. W. J. Morse. This egg was a large membrane-covered egg with a short tubular attachment. A diagram showing its structure is given

in Fig. 4. It contained a normal hard-shelled egg surrounded by a thin layer of albumen[1] which extended into the stalk. The pointed end of the included egg lay toward the stalked end of the including egg. The including egg did not show a perceptible pointing of the end opposite the stalk but several distinctly pointed stalked eggs have been observed at this laboratory. In all cases the stalk occurred at the blunt or air cell end of the egg. Two stalked and pointed double eggs have occurred (see Plate I. and Text-fig. 5) and in these cases also the stalk was at the blunt end. Further eggs have been found in the oviduct which had not rounded off completely at the end toward the funnel but were trailing a stalk of albumen. In no case has an egg been observed in the duct which was not rounded off at the end which was toward the caudal end of the duct. A few words in regard to the processes involved in shaping the egg seem necessary at this point.

Before the egg receives its egg membrane it is a fluid body which tends to take a spherical form. It becomes elongated in the direction of the long axis of the oviduct due to the fact that the diameter of the oviduct is smaller than the diameter of the egg if it maintained a spherical form. The outline of an egg is sometimes nearly elliptical indicating that the duct did not offer much resistance to the egg when the peristaltic action of the duct walls forced it forward. More often, however, one end of the egg is distinctly more pointed than the other. This distinction between the two ends is seen in many of the membrane-covered eggs found in the isthmus at autopsy. It seems probable that the relative tension of the longitudinal and circular fibers of the duct wall at the time the egg receives its membrane is the most important factor in determining the shape of the egg. If the longitudinal fibers in the wall of the duct ahead of the egg do not contract enough considerably to enlarge the duct as the contraction of the circular fibers behind forces it forward, the egg will meet with considerable resistance and will tend to become pointed. In all cases observed where an egg in the isthmus had its poles differentiated the pointed

[1] As the egg had been preserved in 70 per cent. alcohol, the albumen was coagulated and its normal consistency could not be observed.

end of the egg lay toward the caudal end of the duct. So far as we know if an egg becomes pointed the pointed end is always the end away from the funnel end of the oviduct. For convenience we may speak of the end which first passed down the duct as the anterior end and the opposite end as the posterior end of the egg. In an egg which is distinctly pointed the anterior end is the pointed end and the blunt end is the posterior end. From the facts cited in the preceding paragraph it is practically certain that the presence of a stalk attached to one end of an egg also differentiates the poles, the stalked end being the posterior end.

In specimens 6 and 7 the anterior and posterior end of both included and including eggs were differentiated. Both included and including egg of specimen 6 were pointed. The including egg of specimen 7 was stalked. In both 6 and 7 the anterior (pointed) end of the included egg lay toward the posterior (blunt or stalked) end of the including egg. Parker pointed out that the location of the pointed end of the included egg at the pointed end of the including egg indicates that the included egg has been forced up the duct without reversing its poles. Patterson (13) describes a double egg in which the long axis of the enclosed egg meets the long axis of the enclosing egg at an oblique angle. "On account of this inclination of the enclosed egg its pointed end lies nearer to the blunt than to the pointed end of the enclosing egg." In regard to the significance of this unusual orientation Patterson says "This unusual position of the enclosed egg doubtless has been brought about by crowding and does not indicate necessarily that it was at first incorrectly oriented." However, the observance of cases 6 and 7, where the anterior end of the enclosed egg lies toward the posterior end of the enclosing egg, indicates that the reversal of the poles of the enclosed egg sometimes occurs. The small diameter of the oviduct when compared to the length of the long axis of the egg raises the question, How can this reversal take place?

It has been stated that when a pointed egg was found in the isthmus in all observed cases the pointed end was caudad. This position in reference to the axes of the duct was also maintained by a large per cent. of the eggs which have been found in the

shell gland at autopsy or have been found partly extruded from the cloaca in cases of egg-bound birds. However, we have observed a few cases where the egg in the shell gland or partly extruded was blunt end caudad. Moreover, a series of observations carried on at this laboratory confirm Bonnet's (1) statement that the pointed end of the egg is usually laid first but that occasionally the blunt end comes out first.

Since so far as we know the egg is always formed with its pointed end caudad but is sometimes laid blunt end first it seems probable that in the latter cases the egg turns in the duct. In this connection the results of some rough preliminary experiments in an investigation of the mechanism of laying are of interest. During routine autopsy work hard-shelled eggs found in the shell gland were forced out by pressure from behind. Usually the eggs passed directly backward and out pointed end

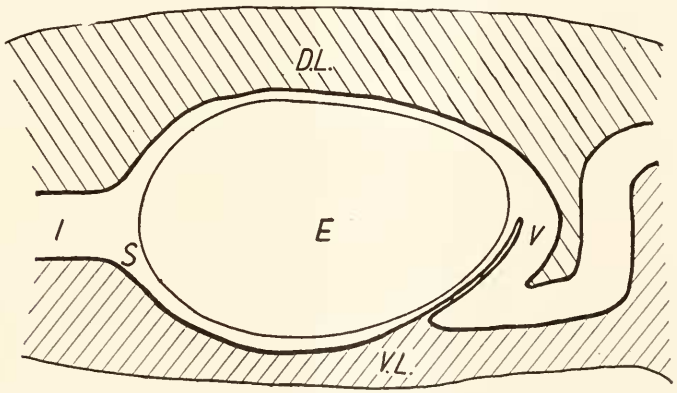


FIG. 5. Diagram showing an egg in the shell gland. *D.L.* = Dorsal ligament; *E* = egg; *I* = isthmus; *S* = shell gland; *V* = vagina; *V.L.* = ventral ligament.

first, but several times the pointed end turned dorsad and the egg reversed ends. Continued pressure then forced the egg out blunt end first. Fig. 5 shows a diagram of the egg in the uterus.

If pressure is so applied that the pointed end is pressed directly into the opening of the vagina or ventral to it on the thin fold the egg comes out pointed end first. However, if it is pressed against the uterine wall slightly dorsal to the vaginal opening, the point slips along this curved caudodorsal angle of the uterus

and the egg reverses ends. In the region of the uterus the ventral ligament becomes a thick band of muscle fibers from which fibers extend over the uterus, some of them reaching the caudo-dorsal angle of the body wall. This band of muscles is no doubt an important, perhaps the chief mechanism concerned in extruding the egg. Since it is heaviest on the ventral side of the uterus and since fibers connect it to the body wall dorsal to the opening of the vagina, it seems reasonable that their contraction in normal laying may sometimes bring the point of the egg against the uterine wall dorsal to the opening of the vagina and that in such a case the egg reverses ends.

In cases 6 and 7 the reversal of the axes of the included egg may have occurred in this manner and then a change in direction of the muscular action of the duct may have forced the egg back up the duct pointed end first. In the albumen-secreting region the direction was again reversed. In each of these cases the included egg appeared to be the only nucleus of the including egg and it seems probable that it furnished the necessary stimulus to cause the secretion of the including egg envelopes. There was no membrane immediately surrounding the included egg. Evidently it did not acquire one going up.

Specimens 8 and 9 were found in oviducts during routine autopsy work. They show the processes of double egg formation in actual operation. Specimen 8 was found in the albumen-secreting region with its posterior end 10 centimeters from the beginning of the isthmus. The included egg was surrounded by a layer about one half cm. deep of very thick albumen. Underneath this was a firm egg membrane within which was a normal hard-shelled egg surrounded by a layer of thick and one of thin albumen. Evidently this egg had been forced from the uterus up the duct to the albumen-secreting region. Since there was no membrane around the egg inside the albumen envelopes it could not have received a membrane going up. In the albumen-secreting region its direction was again reversed and it evidently stimulated the secretion of albumen and egg membrane. It was then again returned into the albumen-secreting region where it again stimulated the secretion of albumen. In this condition it was found at autopsy.

Specimen 9 was found with the craniad or blunt end of the included egg 14 centimeters from the funnel mouth. The normal hard-shelled egg had a shell membrane around the shell. There was no albumen between the shell and this membrane. Either the egg had received this membrane on its way up or it had been carried into the albumen-secreting region and immediately carried back into this isthmus and after receiving the membrane had been carried back up the duct nearly to the funnel mouth. This had probably happened only a short time before death, since there was no albumen formed around the outer shell membrane.

A diagram of specimen 10 is shown in Fig. 6. From the

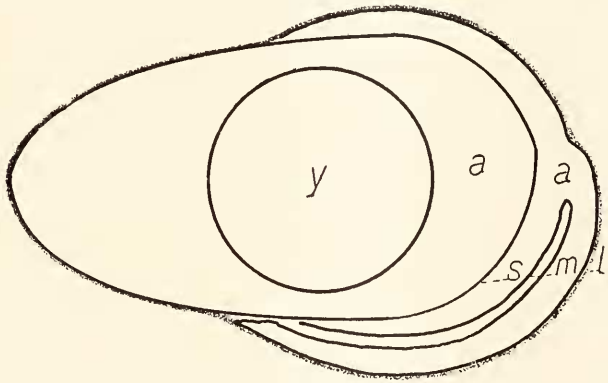


FIG. 6. Showing the structure of double egg No. 10. *a* = albumen; *l* = granular shell material; *m* = egg membrane; *s* = shell; *y* = normal yolk.

figures it may be seen that the enclosed hard-shelled normal egg is not entirely surrounded by all of the envelopes of the enclosing egg, but the second egg membrane filled with albumen forms a cap which covers the blunt half of the enclosed egg. At the posterior pole of the egg the enclosing cap of egg membrane is continued into a stalk also filled with albumen. This stalk is folded down against the side of the egg. The whole egg is covered with a continuous layer of granular shell material. The stalk of the including egg must have been folded down before this shell was secreted as the under side of the stalk and the part of the egg membrane against which it lay were free from shell.

In this case also it is apparent that a normal distinctly pointed hard-shelled egg was forced back up the duct without reversing its poles. The question of where the direction was again reversed is less easily decided. The fact that the pointed half is not covered with membrane suggests that it was forced only half way into the isthmus, this would not account for the presence of thick albumen in the cap and stalk. While Pearl and Curtis (13) have shown that albumen is secreted in the isthmus and uterus, they have seen no evidence of as thick gelatinous an albumen as that found in this egg outside of the albumen-secreting region. It would seem that the egg had been forced back part way into the albumen-secreting region and had either stimulated the formation of albumen or had met a stalked dwarf egg coming down. Why in this case membrane was not formed around the whole enclosed egg is difficult to say. While it is possible that some albumen is necessary to cause the secretion of an egg membrane, case 9 above and two cases described in an earlier paper (4) have shown an egg membrane closely applied to a hard-shelled or a membrane-covered egg with no visible albumen between them.

Another peculiarity of this egg is the position of the stalk. Stalked eggs while infrequent are a well-known type of abnormal eggs. The stalk is usually continued straight in the long axis of the egg. Sometimes it is more or less coiled or crushed down onto the blunt pole of the egg and sometimes in this position it becomes covered with shell and forms a projection more or less resembling a snail shell. How pressure from behind could cause the straight folding down seen in specimen 10 is hard to imagine. If the egg immediately on entering the uterus had its poles reversed in the manner described on page 191 this position of the stalk would be the natural result. If this is the explanation, the reversal of poles must have occurred before the shell was formed. The egg must then have remained in the uterus for some time before it was laid.

(*b*) *The Enclosed Egg Was a Dwarf Egg in Specimens 11, 12, 13, 14, 15 and 16.*

Specimen 11 was a soft-shelled dwarf egg which weighed 11.1 grams. When this egg was opened it was found to contain a

small hard-shelled dwarf egg surrounded by very thick albumen. This small egg had a short stalk which was open at the end. The enclosed egg including the stalk was filled with a clear thick albumen. The stalked end of the enclosed egg lay toward the blunt end of the enclosing egg. Evidently this short-stalked dwarf egg had been returned from the shell gland to the albumen-secreting region without reversing its poles and without acquiring an egg membrane going up. It had then evidently furnished the stimulus for the formation of the enclosing envelopes.

A photograph of specimen 12 is shown in Plate III., Fig. 2. This egg was much like specimen 11, but was much larger. The complete egg weighed 32.0 grams. Both enclosed and enclosing eggs had hard shells. The enclosed egg contained a mass of chalazal fibers surrounded by rather thin albumen. One end of this egg was contracted to a short stalk-like portion. There was a circular area at one side of the end of this stalk-like appendage which was not covered with either membrane or shell. That is, the albumen was exposed. The enclosed egg weighed 7 grams. Externally it was lightly covered with a mass of chalazal-like fibers which projected from the poles into the albumen. In the mass at the finished end was a small drop of yolk and a small lump of hardened albumen. Surrounding the central mass formed by the dwarf egg, yolk drop and lump of hardened albumen with their wrapping of chalazal-like threads were layers of thick and thin albumen. The unfinished end of the included egg lay toward the blunt end of the including egg. Evidently without turning around the enclosed egg had backed up the duct nearly to the funnel mouth. It had not acquired an egg membrane going up. It had there united with the drop of yolk and the lump of hardened albumen. Either these particles or the included egg, or both together, had furnished the stimulus for the secretion of the egg parts of the including egg.

Specimens 13, 14 and 15 are so nearly alike that one description will suffice for them all. These eggs varied in weight from 12.5 to 15.0 grams. Each egg was hard-shelled. A diagram of No. 13 is given in Fig. 7. With slight modifications in the size and shape of the irregular mass of yolk this diagram would represent equally well either of the other two eggs. In each of

these cases the enclosed egg was a membrane-covered egg without any visible distinction between the poles. In each case the enclosed egg contained a small irregular mass of yolk wrapped in a mass of chalazal-like threads and surrounded by albumen. In specimens 13 and 14 all of this albumen was thick, but in specimen 15 both thick and thin albumen were present. In each case the included egg had at each pole a bunch of chalaza-like fibers resembling imperfect chalazæ. In each case the

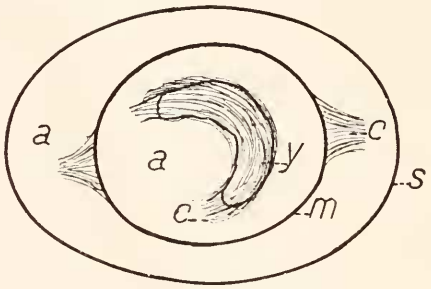


FIG. 7. Diagram showing the structure of double egg No. 13. *a* = albumen; *c* = chalazal-like threads; *m* = egg membrane; *s* = shell; *y* = yolk.

including egg contained both thick and thin albumen. Evidently in each case a dwarf egg which contained a small amount of free yolk was returned from the isthmus to the uppermost part of the oviduct and there furnished the stimulus for the formation of the including egg.

Specimen 16 is somewhat similar to the three eggs just described. In this case the included egg did not contain yolk. Neither were there visible chalazæ at its poles. That is, a yolkless membrane-covered dwarf egg was evidently returned to the albumen-secreting region where it furnished the nucleus for the including egg.

BRIEF DESCRIPTION OF FOUR DOUBLE EGGS FOUND IN THE BODY CAVITY OF A BIRD WITH AN ABNORMAL OVIDUCT.

Beside the 16 specimens described above, 4 specimens were found in the body cavity of a bird with an abnormal oviduct. The case has been described in a previous paper (4). "The oviduct was perfectly normal from the funnel mouth to the posterior end of the isthmus where the tube ended blindly.

There was no shell gland or vagina." This bird had a normal ovary and the oviduct functioned as far as it was developed. The eggs were then backed up the duct into the body cavity where they were absorbed. A large number of empty egg membranes and 14 eggs in every stage of absorption from a normal fresh membrane-shelled egg to empty egg membranes were found in the body cavity at autopsy. While most of these eggs had apparently been normal, 4 of them were double eggs. In one of the four the enclosed egg was also a double egg. Another one was made up of a concentric series of four enclosed eggs. A brief description of these four eggs is given in the original paper.

Discussion.

The occurrence of double eggs is not infrequently noted in the agricultural journals. Such eggs have usually been mistaken for normal or double-yolked eggs until broken for domestic use. The observations on the arrangement of the contents is, therefore, usually not noted. When descriptions of the arrangement were attempted they have for the most part agreed with those described above. E. W. Pick (11), however, describes a double egg in which he states that the normal yolk was in the "tapered or smaller end of the egg, while in the 'bell' or larger end there nestled a smaller egg, shell intact." So far as we know in all other cases observed where an egg is enclosed in another egg which contains a yolk the yolk is in the blunt or bell end. The egg described by Mr. Pick was supposed to be "an ordinary double-yolked egg" until "broken for domestic use." Since it is necessary to observe very carefully the arrangement of the parts of an egg before they are taken from the shell in order to be sure the positions have not been disturbed it seems possible that in this case the record may not be accurate. In specimens 1 and 2, however, a common egg membrane enclosed a normal hard-shelled egg and a naked egg composed of a normal yolk surrounded by albumen. These two eggs lay with their long axes parallel. At least in specimen 1 the evidence is convincing that the two eggs passed through the isthmus side by side. If two eggs can come side by side as in these cases it is at least possible to conceive that they may occasionally pass. In such a

case the reversal of the direction of the upward moving of the hard-shelled egg after it had met and passed its successor might result in a double egg with a yolk in the pointed end and a hard-shelled egg in the blunt end.

Parker (12) cites cases of "soft-shelled" eggs in the body cavity described by Davaine (5) and Landois (10). Several other cases are described by von Durski (6). A few cases of membrane-covered or normal hard-shelled eggs in the body cavity of apparently normal birds have been observed by the authors. A previous description (3) of a bird which backed all her eggs into the body cavity due to the fact that the oviduct ended blindly at the posterior end of the isthmus has been briefly summarized on page 196. It has also been shown by Pearl and Curtis (15) that eggs are found in the body cavities of birds killed some months after their oviducts have been ligated in the isthmus or shell gland. If the ligature is in the isthmus the eggs are membrane-covered but if all or part of the shell gland lies above the ligature some or all of the eggs have shells. Since the egg membrane and shell are formed in the caudal portion of the oviduct and since in all the above cases where eggs were found in the body cavity the funnel mouth was the only opening of the oviduct into the body cavity, the egg must have been returned up the duct and out through the funnel mouth after having passed as far as the isthmus or shell gland. In two cases the authors have observed hard-shelled eggs with no secretion around them in the upper portion of the albumen-secreting region. Specimens No. 8 and 9 described above were also hard-shelled eggs found in the albumen-secreting region of the duct. In these four cases also the eggs must have been returned from the shell gland.

So far as we know an egg has never been observed moving up the duct nor have any movements of the duct been observed which would tend to force an egg in that direction. It has been generally assumed (Parker 12, von Durski, 6, Hargitt 8, Patterson 13, and Pearl and Curtis 15) that the backward movement of the egg is due to antiperistalsis. In the present paper no assumption has been made as to the nature of the muscular action. It seems possible that this may take some other form

than antiperistalsis. C. J. Hick and J. W. Visher (9) have lately analyzed the muscular movements which cause regurgitation of the duodenal contents into the stomach and have found that they are not antiperistaltic in character. The precise nature of the movements of the oviduct which force an egg backward can be more safely decided after they have been observed or experimentally produced. An examination of the cases discussed in the literature and described above give little evidence that the oviduct glands are excited to pour out their secretion by an egg moving up the duct. If this were the case a complete normal egg in the body cavity or in the upper part of the oviduct or included within another egg would have a reversed set of egg envelopes surrounding the shell. That is, around the shell would be an egg membrane. This membrane might be separated from the shell by thin albumen since it has been shown (14) that this albumen is formed in the isthmus and uterus and taken into the egg through the egg membrane probably by osmosis. Whether or not thin albumen can come in after the thick albumen is covered by a completed shell is not known. The egg normally comes to its full weight before the shell is very thick. Surrounding the egg membrane we would then find a layer of thick albumen. This is not the case in most eggs which have backed up the duct. The eggs found in the body cavity and upper oviduct as a rule were in the same condition as an egg from the lower part of the duct. In case they were included or were becoming included in a second set of egg envelopes these envelopes were in the same oder in the including as in the included egg. That is, albumen, egg membrane and shell. This indicates that the envelopes were formed during a second passage down the duct. In this connection Hargatt (8) says: "If it should be queried why such deposition might not have taken place on the ascent of the egg by antiperistalsis as well as during its later descent, it may suffice to admit that perhaps it did occur. However, in case the return of the egg up the oviduct took place soon after its original descent, the glandular structure would be in a state of exhaustion and hence capable of only slight discharge." However, it has been shown in earlier investigations from this laboratory (2) that the passage

of a normal egg does not exhaust even temporarily the oviduct glands since both albumen and shell are heavier in double-yolked than in single-yolked eggs, while both parts are still heavier in triple-yolked than in double-yolked eggs. It was further shown (3) that in many cases the second yolk must have followed the first quite closely since a normal egg was produced on the day preceding the day on which the multiple-yolked egg was laid. The time between the passage of these yolks must have been less than the time required to form a complete hard shell. The very plausible explanation offered by Hargitt does not, therefore, seem tenable. Two other suggestions may be made but neither of them can be proven at present. One is that perhaps the oviduct is polarized to such an extent that secretion is discharged only when the stimulus advances in the normal direction. The other is that perhaps the egg moves very rapidly up the duct and that there is not sufficient time for the stimulus to become effective. That the egg does move rapidly up the duct is suggested by the fact that in the birds with ducts ligated in the shell gland the eggs with complete shells are forced up the ducts before the succeeding yolks enter the funnels. Normally ovulation takes place very soon after laying.

It should, however, be stated that there are a few cases known where some portion of the duct has for some reason failed to form its normal secretion around an egg which has passed in the normal direction. The two cases which have come under our personal observation may be briefly cited. A photograph of the egg produced in the first case is shown in Plate III., Fig. 3. When the egg was found in the nest the two parts were pressed together over the dark areas which face each other in the photograph. They were held together only by the thin layer of shell which covers all of the two egg membranes except the approximated portions. This shell cracked off when the egg was handled and the circular areas free from shell were exposed. The two eggs had apparently been flattened together but the membrane rounded up as they separated. The egg shown at the right was a normal egg with normal yolk, albumen and egg membrane. The other egg was a normal yolk surrounded by an egg membrane. This egg contained no visible albumen. When

the egg membrane was cut and stripped off the clean normal vitelline membrane was exposed. This egg must have passed through the albumen region of the duct without receiving any perceptible quantity of albumen. Whether this was due to exhaustion of the albumen glands or to a rapid passage of the yolk or to some other cause is not known. Evidently under certain conditions the albumen glands do not respond to their normal stimulus even when passing in the normal direction. A failure of the albumen glands is not necessarily accompanied by a failure of the membrane-secreting glands of the isthmus. This egg evidently overtook the normal egg after it had received its egg membrane but before any shell was formed.

In the other case an apparently normal bird laid an egg which consisted of a normal yolk surrounded by albumen but without either membrane or shell. That is, the membrane- and shell-secreting glands failed to respond to their normal stimulus. In this case also the cause for this failure is not known.

In spite of what seems to be a general rule that including egg envelopes are formed only during a downward passage of the egg, three cases are known in which it is possible that the glands of the isthmus may have been stimulated to the secretion of an egg membrane by an egg passing up the duct. Specimen 9 was a hard-shelled egg surrounded immediately by an egg membrane and was found in the upper albumen-secreting region of the oviduct. As previously suggested, this may have received the egg membrane when passing up or it may have been returned from the lower albumen-secreting region to the isthmus and then again been forced up the duct to the position in which it was found. However, it was more than half way up the albumen-secreting region and had not yet received any albumen. Also two of the double eggs found in the body cavity of the bird with the congenitally closed oviduct had an egg membrane closely surrounding the enclosing egg. In either of these cases the enclosed egg may have received the membranes going up or it may have been temporarily moved caudad from the lower albumen region to the isthmus. In either of these cases it is also possible that there had originally been some albumen between the enclosed egg and the enclosing membrane which had been absorbed.

In a previous investigation (3) attention was called to the possibility that under certain conditions the return of an egg up the duct may result in the formation of a double-yolked egg. It seems quite possible that the reversal of the direction of an egg may be more frequent than we have formerly supposed. The result of such a reversal must depend on the state of development of the egg when the backward motion begins, the extent of the backward movement, the rate of fecundity of the individual bird at the time, etc. For example, if an egg which has not yet received its egg membrane is forced backward toward the funnel but not expelled from the duct and then without meeting its successor moves forward again the only result will be an unusually large amount of albumen. If an egg without a membrane which is moved up the duct but not expelled meets its successor and returns with it through the duct, the result will be a double-yolked egg. While it must be admitted that since the succeeding yolk is not usually ovulated for some hours after an egg has received a membrane, yet yolks are certainly sometimes ovulated at considerably shorter intervals. It is also possible that the tone of the oviduct may sometimes be so low that an egg may remain practically stationary for a time. So soon, however, as the egg receives its membrane it can combine with its successor only as a double egg. Either a normal or a dwarf egg may be returned up the duct and may either be expelled from the funnel into the body cavity or at any level of the duct the direction may again be reversed. If the egg becomes united with its successor it becomes enclosed with it in some common envelopes, the number which are common depending on the level of the duct at which the components unite. If it does not meet its successor it becomes a nucleus around which are formed the envelopes which are normally secreted below the point where the forward direction is again resumed. It thus seems that the formation of a double egg does not involve unique processes, but that this phenomenon results from certain combinations of processes, most of which are the normal processes of egg formation. The abnormal factor—the reversal of direction of the egg—when of greater extent results in the expulsion of the egg into the body cavity from which it is

usually absorbed without difficulty. When the reversal of direction is of less extent the result may be a normal egg with a large percentage of albumen or rarely it may be a double-yolked egg.

Summary.

1. A membrane-covered or hard-shelled normal or dwarf egg may be returned up the duct and may either meet its successor and return with it, becoming enclosed in a common set of egg envelopes, or not meeting its successor it may again be forced through the duct stimulating the secretion of a set of egg envelopes around itself.

2. The number of egg envelopes common to the enclosed egg and the yolk of the enclosing egg or the number of egg envelopes which surround the enclosed egg when the enclosing egg has no yolk depends apparently on the level of the duct at which the enclosed egg resumes its normal direction toward the cloaca.

3. The enclosed egg is usually forced up the duct without turning on its axis but occasionally the poles are reversed.

4. A similar reversal of poles sometimes occurs in normal laying and it seems probable that in both cases this turning takes place in the uterus when the first powerful contractions of the uterus brings the outwardly directed end of the egg slightly above the opening from the shell gland into the vagina and tangentially against the curved caudo-dorsal angle of the uterus.

5. The enclosed egg usually precedes its successor through the duct and, therefore, usually lies in the pointed or anterior end of the enclosing egg, while the yolk of the enclosing egg lies in the blunt or posterior end.

6. However, in two known cases where the enclosed egg united with its successor after the latter had received practically all its thick albumen there is evidence that the two eggs came side by side in the duct with their long axis parallel and in one case they certainly passed through the duct side by side with their long axes parallel to each other and also parallel to the long axis of the duct.

7. There has been one case described with the yolk in the pointed and the enclosed egg in the blunt end of the enclosing egg. There is some doubt about the accuracy of this observation but it is possible that two eggs can pass in the duct.

8. A hard-shelled egg uncovered by membrane or albumen is sometimes found in the body cavity or upper oviduct while a hard-shelled egg enclosed within another egg is not usually immediately surrounded by an egg membrane. It would, therefore, seem that the egg does not cause the secretion of egg envelopes around itself on its way up the duct.

9. Since in the case of a double-yolked egg a second yolk closely following the first does stimulate the secretion of the successive envelopes, it does not seem probable that the failure of the duct to form envelopes around the returning egg is due to exhaustion of the glands.

10. The reason for this failure is not known. It may be that the return of the egg is very rapid and that the time of application of the stimulus is too short to be effectual, or there may be a real polarity of the duct so that it responds only to a downwardly directed stimulus.

11. A few cases are known where one or more of the normal egg envelopes have not been formed around an egg advancing in the normal direction (for example, a yolk enclosed by egg membrane and shell but with no albumen, or a laid egg composed only of normal yolk and albumen). The cause for these phenomena are not known. In these cases the movement of the egg may have been abnormally rapid.

12. The occurrence of membrane-covered or hard-shelled eggs in the body cavity, the albumen-secreting region of the oviduct or enclosed within the albumen of another egg shows that an egg may be moved up the duct, but since an egg has never been observed moving in this direction the nature of the motion can only be imagined.

13. The double egg results from a modification of the normal processes of egg formation due chiefly to a reversal in the direction of the egg after it has received its membrane or its membrane and shell. This backward movement must cease before the egg is expelled from the funnel mouth and the movement in the normal direction must be resumed.

14. If the backward movement sets in before the egg receives its membrane but stops before it is expelled from the funnel mouth and if the normal direction is then resumed, the result

will be a normal egg with a large percentage of albumen or in case the returned egg meets its successor, a double-yolked egg.

15. If the backward movement of an egg in any stage does not stop too soon, the partly or fully formed egg will be expelled from the funnel mouth into the body cavity.

16. In case the oviduct is naturally or artificially closed the eggs are regularly expelled by forcing them out the funnel mouth.

17. A double egg then is the result of a combination of normal and abnormal processes which when combined in other proportions result in other abnormal phenomena of egg production.

18. An egg may move backward and forward several times in the duct as is shown by the production of an egg enclosed within a series of concentric egg membranes separated by thick albumen.

LITERATURE CITED.

1. **Bonnet.**
'83 Das Vogelei. Deutsche Zeitschrift für Thiermedicin, Vol. IX., pp. 239–252.

2. **Curtis, M. R.**
'14 Studies on the Physiology of Reproduction in the Domestic Fowl. VI. Double and Triple Yolked Eggs. BIOL. BUL., Vol. XXVI., pp. 55–83.

3. **Curtis, M. R.**
'15 Studies on the Physiology of Reproduction in the Domestic Fowl. XI. The Relation of Simultaneous Ovulation to the Production of Double-yolked Eggs. Jour. Agr. Research, Vol. III., pp. 375–386.

4. **Curtis, M. R.**
'15 Studies on the Physiology of Reproduction in the Domestic Fowl. XII. On an Abnormality of the Oviduct and Its Effect upon Reproduction. BIOL. BUL., Vol. XXVIII., pp. 154–162.

5. **Devaine, C.**
'61 Mémoire sur les anomalies de l'oeuf. Compt. Rend. et Mém. Soc. Biol., Paris, Sér. 3, T. 2, pp. 183–266.

6. **von Durski, S. P.**
'07 Die pathologischen Veränderungen des Eies und Eileiters bei den Vögeln. Inaugural-Dissertation. Berlin.

7. **Gruvel, A.**
'02 Sur un cas spécial d'oeuf tératologique. Proc. Verb. Soc. Sci. Phys. Nat. Bordeaux Ann., 1901–1902, pp. 72–76.

8. **Hargitt, C. W.**
'12 Double Eggs. American Naturalist, Vol. XLVI., pp. 556–560.

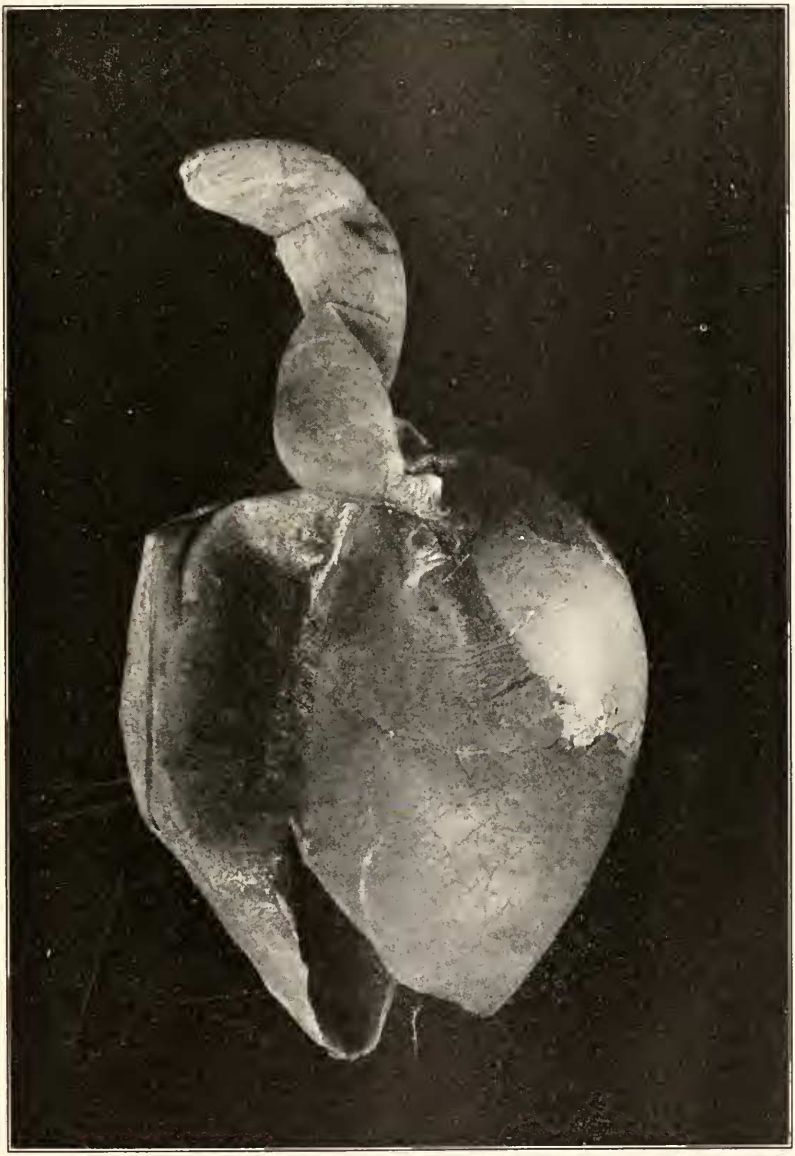
9. **Hicks, C. J. and Visher, J. W.**
'15 Contributions to the Physiology of the Stomach. XXVII. The Mechanism of Regurgitation of Duodenal Contents into the Stomach. Amer. Jour. of Physiol., Vol. XXXIX., pp. 1–8.

10. **Landois, H.**
'99 Hühner-Eier in der freien Bauchhöhle. 27 Jahresb Westfalischen Prov.-Vereins f. Wiss. und Kunst, 1898–99, pp. 52–53.
11. **Pick, E. W.**
'11 Egg Abnormalities. Poultry World (London), Vol. 7, p. 495.
12. **Parker, G. H.**
'06 Double Hens' Eggs. American Naturalist, Vol. XL., pp. 13–25.
13. **Patterson, J. T.**
'11 A Double Hen's Egg. American Naturalist, Vol. XLV., pp. 54–59.
14. **Pearl, R. and Curtis, M. R.**
'12 Studies on the Physiology of Reproduction in the Domestic Fowl. V. Data Regarding the Physiology of the Oviduct. Jour. Exper. Zool., Vol. 12, pp. 99–132.
15. **Pearl, R. and Curtis, M. R.**
'14 Studies on the Physiology of Reproduction in the Domestic Fowl. VIII. On Some Physiological Effects of Ligation, Section, or Removal of the Oviduct. Ibid., Vol. 17, pp. 395–424.

DESCRIPTION OF PLATES.

PLATE I.

Photograph of double egg No. 1. See description page 182.



MAYNIE R. CURTIS.

PLATE II.

Photographs of double egg No. 2. Fig. 1, outside view. Fig. 2, view after outer membrane was opened.

FIG. 1.

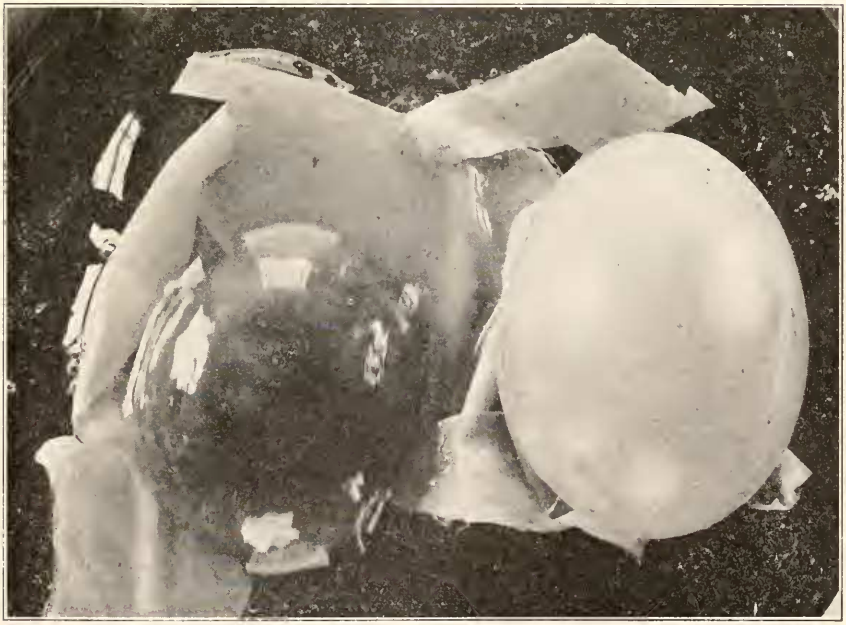


FIG. 2.

PLATE III.

FIG. 1. Contents of dwarf egg No. 3. Note normal yolk and hard-shelled dwarf egg which is enclosed only in the outer albumen layers of the normal egg.

FIG. 2. Photograph of the shells of both included and including egg of double egg No. 12.

FIG. 3. Photograph of eggs which were in separate egg membranes but which had been flattened against each other before the shell was deposited so that they were lightly held together by the thin layer of shell which was continuous over their exposed surfaces. Egg at the right was a normal egg. Egg at the left contained a normal yolk but there was no albumen present.

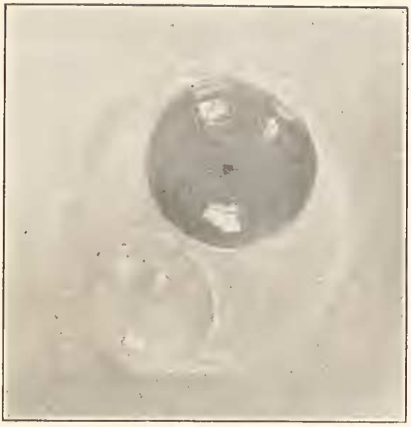


FIG. 1.

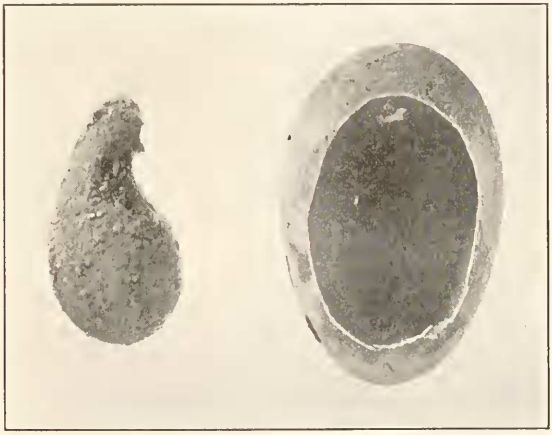


FIG. 2.

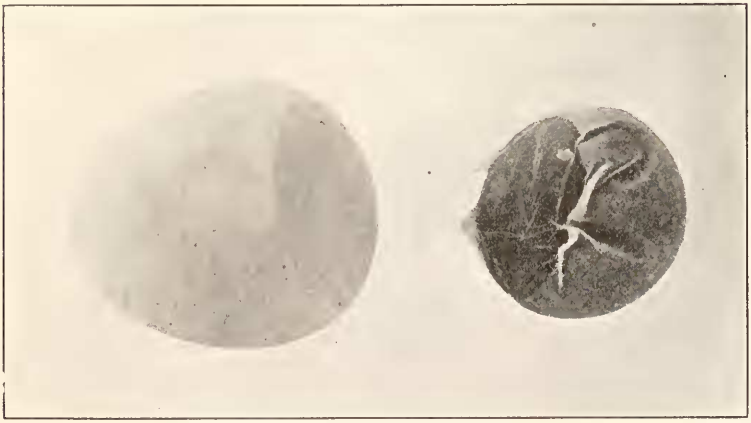


FIG. 3.

NUCLEUS OF CHILOMONAS PARAMŒCIUM EHRENBERG.

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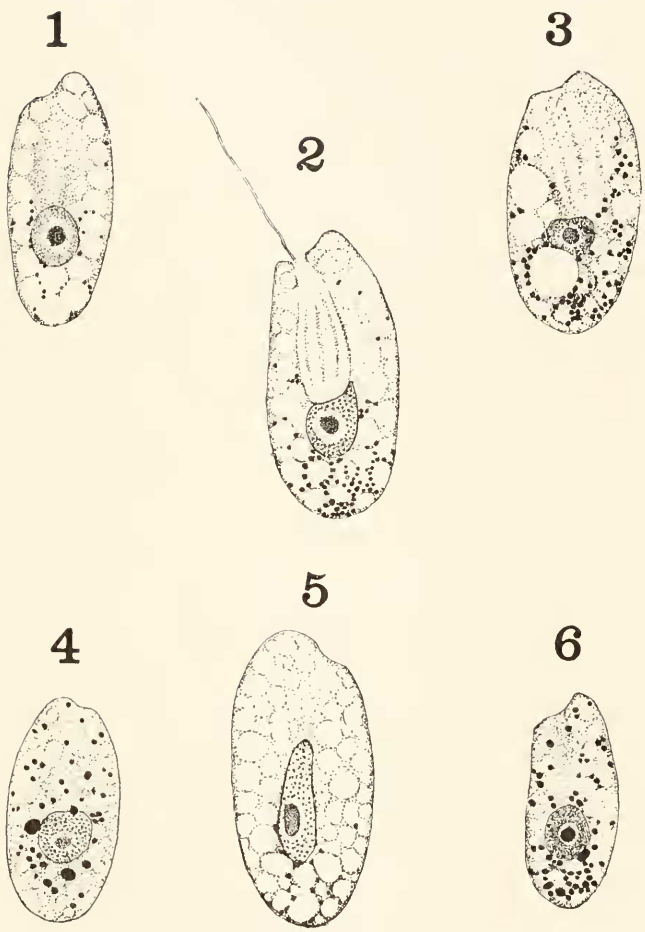
Chilomonas paramœcium Ehrenberg is perhaps the most common flagellate to be found in laboratory aquaria; but the nature of our paper demands that we describe in detail the species with which we are dealing and our methods of preparation.

Our specimens were found in dense swarms in laboratory aquaria that had stood for a certain time. The specimens were taken from the aquarium with a pipette and centrifuged. The infusion-water was then decanted off and the animals were next fixed in aceto-sublimate for one to three minutes and then washed and passed into 70 per cent. alcohol and stained in Delafield's hæmatoxylin. The stained specimens were mounted in dammar. The length of the fixed animals was as much as thirty microns. In life the posterior end of the animal is somewhat pointed and slightly bent dorsally. The anterior end is obliquely truncated from anterior dorsal region posteriorly to ventral side. A pharynx leads from the anterior oblique end of the body well towards or even beyond its middle (Figs. 1 and 2). Associated with this pharynx are certain small rounded bodies arranged in eight rows, each row lying nearly parallel to the axis of the body. These bodies in life appear as highly refractive structures embedded within the wall of the pharynx. Traces of these are not always found in our fixed material. They appear as concentric, arched lines lying parallel to the axis of the body when our fixed material does show them (Fig. 2). Pascher ('13) described these bodies as "kugeligen Körnchen . . . Diese Körnchen entsprechenden der Körnenauskleidung der Furche der niederen Kryptomonaden und rudimentare 'Trichocysten' dar." Bělař ('16) gives a figure of discharged Tapetenkörner or Trickocysten and says: "es scheint hier vielmehr um mehr oder weniger funktionslos gewordene Rudimente der Schleimtricko-

cysten der Cryptomonaden zu handeln." There are also associated with the pharynx two flagella of equal length. Eyferth ('00) says that these arise from the dorsal wall of the pharynx; Jennings ('06, Fig. 72) in his figure does not commit himself as to the place of origin of the flagella. Calkins ('01, Fig. 10, *B*) and Pascher ('13, Fig. 171) show one flagellum arising from the dorsal and the other from the ventral side of the pharynx. A contractile vacuole is within the dorsal anterior extremity of the body. The general cytoplasm is not alveolar, though it usually presents such appearance, because of the presence of colorless, highly refractive spheroidal bodies. It is important to note that these bodies vary in number, and in so far as they decrease in number the apparent alveolar condition of the cytoplasm becomes less evident. We have tested these with the iodine starch test and got a negative reaction—the bodies staining brown. These bodies have the same general optical features as the paramylum grains of *Euglena* and have been described for *Chilomonas* as paramylum grains; they are, therefore, to be looked upon as assimilation products. These assimilation products are more or less directly related to certain deeply staining (nuclear stains) rounded bodies, which lie about them.

Most systematic and experimental workers, when describing *Chilomonas paramœcium* have paid little or no attention to these chromatic bodies. For example, Eyferth ('00), Pascher ('13) and Jennings ('06) neither describe nor depict them. These same authors, however, describe a centralized well-defined nucleus lying behind the middle of the body.

Calkins ('99) recognized no nucleus in this rounded, nuclear-like structure, which lay behind the middle of the body; but he looked upon the deeply staining bodies which lie by the paramylum grains as a distributed nucleus, and thus brought *Chilomonas paramœcium* in line with a series of animals bearing distributed nuclei or permanently granular nuclei. "Forms with this permanently granular chromatin, again, are found in two conditions. In one type the granules are scattered throughout the entire cell, and are never confined by a nuclear membrane (so-called 'distributed' or 'diffused' nuclei). In nuclei of the other type the granules are confined in a definite, more or less



FIGS. 1 AND 5. Specimens with closely packed paramylum grains and few chromatic bodies. × 1,500.

FIG. 2. Shows ventral flagellum, pharynx with rows of structures within its wall—the rudimentary trichocysts. Nucleus is here closely applied to the fundus of the pharynx. Paramylum grains reduced somewhat and the number of chromatic bodies is increased. × 1,500.

FIGS. 3, 4 AND 6. Show the paramylum grains greatly reduced in size and the chromatic bodies greatly increased in size. In these cases the size of both paramylum grains and chromatic bodies varies much. × 1,500.

spherical space, which may or may not be bounded by a nuclear membrane. Examples of the first type were described by Gruber in certain Rhizopods and in a number of Ciliata, and,

as he suggested, it is highly probable that many, if not all of Haeckel's Monera will be found to possess nuclei of this type. Among flagellated forms it has been described by Bütschli ('96) for *Chromatium okenii* and *Ophidomonas jenensis*, and by myself ('98) for a species of *Tetramitus*. In the latter form the granules of chromatin, which at first are scattered throughout the entire cell with no apparent order, come together to form a loose aggregate prior to division. In this condition the aggregate is divided into halves, an equal portion going to each daughter-nucleus (Fig. 2). It is important to note here, however, that another element comes in to complicate the process. In the resting condition of the cells, when the chromatin is distributed throughout the cytoplasm, a faintly staining body can be found somewhere near the center of the cell (Fig. 2, *A*). This body becomes more definite as the chromatin granules come together for division, and it divides into equal portions before the nucleus is halved. During the process of division the chromatin granules become heaped about this partly divided body, one half of which remains in the center of each daughter-heap of granules until the end of the division (Fig. 2, *D–E*). After division the granules again separate, forming the distributed nucleus. The central body, therefore, has the attributes of an attraction sphere.

"The chromatin represented by this temporary aggregation of chromatin granules about the sphere is permanent in the majority of the Flagellates, and may perhaps be regarded as the usual condition of protozoan nuclei. Among some Flagellates the aggregation of chromatin granules about the sphere, although permanent throughout resting and active phases, resembles the loose aggregation of the division period of *Tetramitus* in having no nuclear membrane (*Chilomonas paramœcium*, *Trachelomonas lageuella* and *T. hispida*)." Again Calkins ('01) speaks of what others take to be a nucleus as a division center: "The flagellate *Tetramitus* shows an apparently similar division-center. During the resting phases, the chromatin is distributed throughout the cell, while an indefinite 'achromatic mass' appears to be in direct connection with the cytoplasmic reticulum. Immediately before division, however, the chromatin granules collect about this body, and then, save for the absence

of a membrane, the aggregate resembles the nucleus of *Euglena*. Division takes place as in *Euglena*, the intranuclear division-center dividing first. After division the chromatin granules again disperse and the division-center becomes again cytoplasmic (Fig. 143)." "An intermediate stage between this condition and the condition in *Euglena* is shown by some species of *Chilomonas* and *Trachelomonas* in which there is no nuclear membrane, but in which the chromatin remains permanently aggregated about the division-center." Kellicott ('13), in his statement "a definite nuclear membrane may be absent at first, as in *Chilomonas*," seems to follow Calkins's earlier conception that these granules represent a nucleus without a nuclear membrane.

It is interesting to see that Calkins ('08) modifies his viewpoint somewhat. He no longer considers these granules as representing a nucleus, but implies that they are nuclear in origin. Though it is not exactly clear that he has changed his opinion about these chromatin granules such is strongly implied when he says "there is probably no great difference between the above-described method of idiochromidia formation by transfusion, whereby the chromatin materials percolate through the nuclear membrane in fluid form, and that by nuclear dissolution, whereby the peripheral portion of the nucleus becomes scattered in granular form throughout the cell body" and then in his legend to Fig. 49 on the same page prints "*Chilomonas paramœcium* to show the alveolar structure of protoplasm prior to idiochromidia formation." This idiochromidia he at this place considers to be the "sexual or racial chromatin" of the cell as contrasted with chromidia which is "functionless extranuclear chromatin."

By the implication of his later words he has given up the idea that the nuclear-like body at the base of the pharynx is a nucleus. He now sees, in what he had taken to be the chromatin granules of a distributed nucleus, grains of idiochromidia. These, he says, are of nuclear origin. He further thinks that these chromatic granules play an important part in reproduction.

Our own observations indicate that there is a definite or clearly defined nucleus in *Chilomonas paramœcium*. This

nucleus lies posterior to the middle of the body closely associated with the fundus of the pharynx (Figs. 2 and 3). We have been able to find many of these nuclei in what we take to be the resting condition (Fig. 3). In this condition there is a well-defined nuclear limit indicating the presence of a nuclear membrane. Within the nucleus a single spheroidal nucleolus is seen. This nucleolus in life is highly refractive. The chromatin granules of the resting nucleus are very small and uniform in size. They crowd rather closely upon the nucleolus and leave the nucleolus to lie within a relatively small chromatin-free region (Figs. 3 and 6). In other specimens we find the nuclear size to be increased (Fig. 2) and in some cases the shape of the nucleus, as well, is greatly modified (Fig. 5). In most of these enlarged nuclei the chromatin granules lie more remote from the latter within a relatively large chromatin-free region. The chromatin granules themselves, in such nuclei, are greatly enlarged, though yet uniform in size. These enlarged nuclei we had taken to be prophases in mitosis before the appearance of Bělař's paper ('16) and now we can say that we are able to corroborate in a general way his earlier details for the mitosis of *C. paramœcium*.

Except for these strikingly nuclear-like variations, the character, of what has been termed a "division center," remains constant and we look upon it as the proper nucleus of the cell.

On the other hand, the variability of the extra-nuclear chromatic granules is conspicuous in contrast with the slight variability of the nucleus. These chromatic granules vary in number, size and distribution. We have not found an individual in which no granules are present. These chromatic bodies are smallest when fewest and they then lie within the posterior half of the cell (Figs. 1 and 5). The granules tend to increase in size as they become more numerous (Figs. 2, 3 and 6). They are most widely distributed throughout the cell when they are largest (Figs. 4 and 6). The granules of a given cell, when they are in this last condition, are no longer uniform in size (Figs. 4 and 6).

Associated with this variability of the chromatic bodies we have a variability of the paramylum contents of the cytoplasm. The granules are fewest and smallest when the paramylum bodies are relatively most numerous and when the latter lie

closely packed within the cytoplasm (Figs. 1 and 5). As the paramylum granules become reduced in size the chromatic bodies become more frequent and lie within the enlarged masses of cytoplasm that fill the interstices between the paramylum grains (Figs. 2 and 3). Finally the granules of chromatic material are largest, most numerous and most widely distributed when the paramylum grains have been reduced to small, widely separated spheroids (Figs. 4 and 6).

These chromatic bodies, moreover, arise in the cytoplasmic interstices between the paramylum grains. They lie closely applied to the paramylum elements rather than to the nucleus. Their contact with the nucleus seems to be purely incidental. They appear rather to be bodies related to the changes involved in the assimilation of the paraymlum grains. Their origin, then, we consider to be cytoplasmic and not nuclear. These chromatic granules we take to represent by-products or intermediate phases of the assimilation of paramylum and are not idiochromidia.

Conclusions.

1. There is a well-defined nucleus with a definite nuclear membrane in *Chilomonas paramœcium*.

2. The chromatic bodies, that are found within the cytoplasm, are related to the formation and disposal of paramylum and must not be considered to be idiochromidia, for they are cytoplasmic and not nuclear in origin.

LITERATURE.

Bělař, Karl.
'16 Protozoenstudien. II. Arch. für Protistenkunde, Bd. 36, Heft 3.
Calkins, G. N.
'99 Biological Lectures. Woods Holl, 1899.
'01 The Protozoa. The Macmillan Co., New York.
'09 Protozoology. Lea and Febiger, New York.
Eyferth, B.
'00 Einfachste Lebensformen des Tier- und Pflanzenreiches. G. Goeritz. Braunschweig.
Jennings, H. S.
'06 Behavior of the Lower Organisms. Columbia Press, New York.
Kellicott, W. E.
'13 Textbook of General Embryology. H. Holt and Co., New York.
Pascher, A.
'13 Die Süsswasser Fauna Deutschlands, etc. Heft 2, Flagellatæ II. G. Fischer, Jena.

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BIOLOGICAL BULLETIN

STUDIES ON THE CHROMOSOMES OF THE COMMON FOWL AS SEEN IN TESTES AND IN EMBRYOS

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Some years ago ('09*b*) I gave an account of the spermatogenesis of the common fowl insofar as I was then able to interpret it. Since then I have spent much time in further observation, partly on the same but mainly on new and better material. Altogether I have been engaged on the problem at intervals for over ten years. In the aggregate this means many months of continuous work inasmuch as it includes summer months as well as those of the school year. I emphasize the element of time, not because time alone is particularly significant, but rather to show that my problem has not been one worked out as a summer's pastime. And while time is not the chief essential in solving problems in avian spermatogenesis, in my estimation such problems will not be solved without the most painstaking and critical study extending over months of protracted daily observation. The cells are small, the chromosomes tend to mass, and fixation is uncertain. This necessitates the preparation of literally hundreds of slides and then the abandonment of the great majority of these in favor of the few which really show adequate detail upon which it is safe to base conclusions. The latter once secured, however, one has material enough in a single slide to occupy many hours and even weeks of the closest scrutiny.

My later studies tend in the main to confirm my earlier observations. Chief among the latter was the finding of a large curved chromosome, comparable to the so-called sex-chromosome of other forms, which typically passes undivided to one pole of the spindle during the division of the primary spermatocyte.

I have found no reason to reverse my opinion on this point since in all of my preparations in which fixation is adequate, I find in abundance a characteristic chromatic element of constant shape and size which behaves in the manner indicated. I have recorded stage readings of over 900 views of it in my preparations and I have seen and studied many others which it was not deemed worth while to record separately. Although the existence of this element has been called in question (Boring and Pearl, '14), I do not see the least reason to doubt its existence or its constancy. However, as my subsequent account will show, and even as suggested in my earlier report, there is strong reason for doubting that it is a single or univalent element. The outcome of my investigation leads me to believe that it is composed of two curved univalent chromosomes which exist separately in the spermatogonial and somatic cells.

The present account is based upon a study of testicular materials from two Langshan, four Plymouth Rock, and two Rhode Island Red fowls, together with sections of a number of embryo chicks of 9, 10, 13, and 19 days of incubation respectively. Of the embryos, chicks of the 10 and 13 day stage were found to be the most satisfactory and consequently were used most extensively. By the tenth day of incubation the sexes can readily be distinguished and from this time on to the thirteenth or fourteenth day there seems to be an unusually plentiful division of primitive germ-cells in progress.

Methods.

Materials were fixed mainly in Gilson's, Flemming's, Hermann's, and Bouin's fluids. The latter, used straight or with various slight modifications, was perhaps the most universally successful fixing agent. When modified, the alteration took the form of reducing the percentage of acetic acid which tends to swell chromosomes and make them agglutinate more than they would do otherwise, or of the addition of chromic acid which in some preparations proved helpful in getting better definition of both chromosomes and cytoplasm.

In the study of the testicular material, smears were used extensively and as a rule proved more satisfactory than sections.

In making the smear a bit of perfectly fresh, warm testis was minced up into a fine pulp by means of a small-bladed scalpel or with fine scissors and then spread into a thin film between two slides in the same manner that a blood film is prepared. The slides after separation were plunged immediately into the fixing agent where they remained from 30 minutes to several hours depending upon the reagent used. At the end of this time they were washed out in the appropriate liquid and all granules or clumps of tissue which might prevent making a very thin, even preparation were picked or scraped away. From this point the slide was treated in the same way that ordinary sections are treated.

While many stains were tried none was found which surpassed iron-hæmatoxylin for Gilson and Bouin material, or saffranin for tissues fixed in Flemming or Hermann. The hæmatoxylin preparations were usually counterstained with orange G, acid fuchsin or Congo red, although the hæmatoxylin alone was found most satisfactory where photography was attempted. Indeed some of my best preparations were found to be practically worthless for photography because of a vivid red or yellow background which I was unable to eliminate by screens and which therefore prevented adequate contrast in the photograph. In preparations stained with safranin a counterstain of Lyon's blue, lichtgrün, or Gentian violet was commonly employed. I find a one per cent. safranin in anilin water a more satisfactory stain than alcoholic solutions of safranin.

While the hæmatoxylin preparations were far better than safranin preparations for photographing, the latter often revealed more detail in the mitotic figures because of the semi-transparency of the chromosomes which often enabled one to see separate elements where only a continuous black opaque mass would be discernible with iron-hæmatoxylin. Delafield's hæmatoxylin gave satisfactory results with spireme and non-mitotic stages but was of secondary value in the study of the fully formed chromosomes. One set of smears stained first in safranin and later in Delafield's hæmatoxylin proved to be unexpectedly helpful in general study, although unfortunately such material did not lend itself at all to photography.

Aceto-carmine, so frequently recommended for fresh tissues, I found of little value with my material. Its only use was to give information quickly about what stages one might expect to find in his other better fixed material. As one might expect, from the considerable amount of acid in this stain, it quickly swells and distorts chromosomes, and as far as my own preparations were concerned it proved untrustworthy. With the tissue of embryos Bouin's fluid, with and without the addition of chromic acid, was used in the main; sections alone were studied.

At the outset, in the naïveté of my inexperience with photography under the microscope, I had hoped to present much of my evidence in the form of photomicrography, but although I made many attempts it speedily became evident that the utility of the photographic camera would be decidedly limited. In order to show the objects of sufficient size, high powers had to be resorted to and with them the plane of focus became so restricted that details which were plainly to be seen with very little manipulation of the fine adjustment were found to be not at all revealed in the photograph.

However, even with this handicap, I feel that my photographs reveal convincing evidence of certain features which I wish to discuss, and I have therefore used them for Plates I. and II. Most of the pictures were taken with a Zeiss 2 mm. apochromatic objective and a number 8 compensating ocular. In a few instances a No. 4 or a projection ocular was employed. A number of different brands of photographic plates were tried but the Cramer contrast plates proved to be the most satisfactory.

General Scheme of Spermatogenesis in the Fowl.

The general histological structure of the testis of the fowl is in the main similar to that of well-known mammalian forms. The chief differences are the more slender character of the seminiferous tubules and the great reduction in the amount of interstitial cells. Thus, the convoluted seminiferous tubules which contain the germinal cells come to make up almost the entire bulk of the testis.

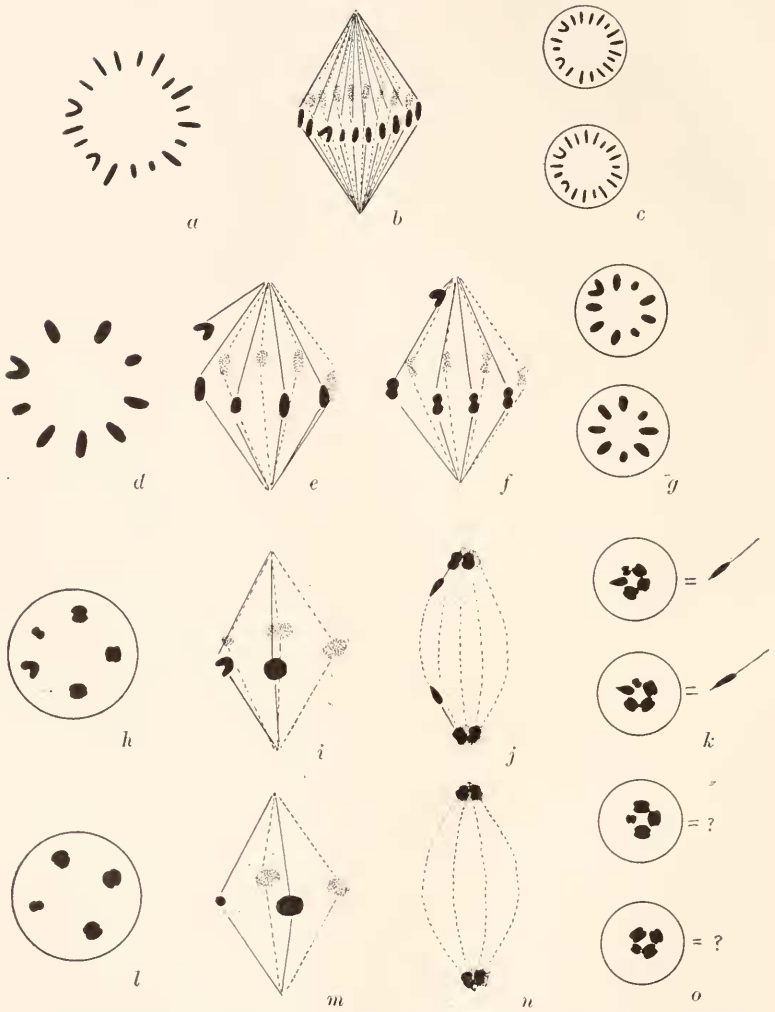
The walls of the tubules are lined by a layer of spermatogonia. These by growth and division give rise to the various later

generations of germ cells which lie inward toward the lumen. As in other well known vertebrates the spermatozoa attach themselves to a Sertoli or nurse-cell for a period before their complete maturation and ejection from the tubule.

The usual four types of cells, (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, and (4) spermatids, are present. After a period of spermatogonial divisions, various of the spermatogonia enlarge to become primary spermatocytes which divide to form secondary spermatocytes. The latter divide again to form the spermatids which ultimately transform into spermatozoa. The spermatocytes, both primary and secondary, and the spermatids, seem to be unattached, or at most, to be very loosely attached in the tubule and they therefore readily spill out onto a slide when the tubules are cut. Consequently it is comparatively easy to get an abundance of these cells for smears. On the other hand, it is very difficult to secure spermatogonia in smears in sufficient numbers for purposes of study. They adhere firmly to the tubule wall and even after mincing with scalpel or scissors are seldom found in any considerable quantity.

As seems general in cases in which the germinal cells are arranged in seminiferous tubules, there appear to be proliferating and resting zones in the same tubule, or possibly some entire tubules are quiescent while others are active. This is evinced by the fact that sections or smears from certain regions of the testis show no active mitoses while others exhibit them in varying degrees of abundance. In still other preparations, mainly spermatids, or spiremes of primary spermatocytes, or some other characteristic stage, constitute the main part of the preparation, as if that special part of the testis were in a particular phase of a general wave of spermatogenesis.

A generalized scheme of the spermatogenesis is shown in Text-figure 1. The spermatogonial chromosomes (*a*, *b*), represented in the diagram as sixteen straight, rod-like chromosomes and two curved chromosomes, divide equationally to produce two sets (*c*) of daughter chromosomes. Details regarding the curved chromosomes are given in later pages. Before the next division, which is that of the primary spermatocyte, the usual



TEXT-FIG. 1. Diagram illustrating the general course of spermatogenesis in the common fowl: *a*, polar view of spermatogonial metaphase showing sixteen chromosomes, of which two are characteristically curved; *b*, spermatogonial metaphase viewed from the side; *c*, products of spermatogonial division; *d*, polar view of metaphase in primary spermatocyte showing nine bivalent chromosomes of which one is U-shaped; *e*, *f*, *g*, successive stages in the division of the primary spermatocyte, the curved chromosome passing undivided to one pole and thus producing a dimorphism in the daughter cells; *h*, secondary pairing of the autosomes at the metaphase of the secondary spermatocytes, the curved element remaining unaffected; *i*, *j*, *k*, stages in the division of the five-chromosomed secondary spermatocytes, each spermatid (*k*) receiving five chromosomes, of which four are probably double and therefore equivalent to eight univalent ones; *l*, secondary pairing of the chromosomes in the secondary spermatocytes which did not receive the curved element; *m*, *n*, *o*, stages in the division of the four-chromosomed secondary spermatocytes, the resulting spermatids (*o*) probably not giving rise to mature spermatozoa.

pairing or synapsis transpires so that nine bivalent chromosomes appear in the metaphase (*d*, *e*) of this mitosis. The curved body, so conspicuous an element of this stage, is regarded as a bivalent chromosome formed through the fusion of the two curved chromosomes of the preceding cycle. In the primary spermatocyte, however, it behaves ordinarily as a single element, passing undivided (*e*, *f*) to one pole of the spindle. In this way two classes of secondary spermatocytes (*g*) are formed one with and one without the curved element.

When the secondary spermatocytes are ready for division, typically only four (*l*) or five (*h*) chromosomes appear in the equatorial plate stage. Inasmuch as the chromosomes are of as large size as those of the primary spermatocytes, and since they entered the secondary spermatocytes as groups of 8 or 9 respectively, the condition found at metaphase in the secondary spermatocytes, I regard as a second fusion of the eight ordinary chromosomes by twos thus producing a class of cells which exhibits four and one which shows five chromosomes (4 + the curved element) at the division time. The double nature of these large chromosomes is often indicated by a bilobed condition (*h*, *l*). Their division, however, is not regarded as resulting in a second reduction in the somatic number of the chromosomes. The division seems rather to result in a halving of each element of such a fused pair. It is not uncommon, in fact, for the daughter elements each still to reveal a bilobed condition as they approach the pole (*j*, *n*), or more rarely to resolve partially or wholly into univalent constituents. The curved element lags at the equator of the second spermatocyte while the other chromosomes are diverging toward the poles but it ultimately divides (*j*), a moiety going to each pole. The spermatids (*k*) formed through division of the five-chromosomed spermatocytes are represented in the diagram as forming spermatozoa, those (*o*) descended from the four-chromosomed spermatocytes are indicated as questionable. The evidence on this point is adduced in a later part of the paper.

Spermatogonia and Male Somatic Cells.

The greatest difficulty experienced in the whole course of study was in securing satisfactory preparations of the spermato-

gonial stages, particularly as regards counts and study of individual chromosomes. The chromosomes are small and usually lie in a web of plasma or linin which takes the same dyes the chromosome do themselves. Furthermore, the chromosomes tend so to stick together and so overlie one another as ordinarily to render individual identification uncertain. As far as my preparations are concerned, it would have been impossible to have come to even an approximately accurate conclusion regarding the number and condition of the chromosomes had I depended on what the spermatogonia of adult fowls exhibit. However, the situation may be alleviated in some measure by using the testes of chick embryos, and I have in large measure resorted to the primordial spermatogonia of such material for my more detailed study, supplementing this also by observations on division stages of embryonic somatic cells, particularly those of the renal tubules.

Even under the best of circumstances, it was difficult to find clear cut cases showing all the chromosomes in one section and so disposed as to render an unequivocal count possible. Most of my notes read "over 16" or "not over 18." The difficulty in the main was that sometimes two or more chromosomes overlapped in such a way that it was impossible to say whether the given object should be counted as one or two or possibly three chromosomes. Inasmuch as the chromosomes are not all of the same size, two of the smaller ones closely apposed might easily be mistaken for one of the larger ones. In general in favorable preparations, one could pick out two to four very small ones, two to four relatively large rod-like ones, two strongly curved ones and the rest rod-like ones of intermediate size.

At the outset it should be said that this finding constantly of a pair of curved elements in the male somatic cells came as a surprise to me. I had carried on my observations so long on the large curved element which is so prominent in the primary spermatocytes that my expectation, in studying the cells of embryonic forms, was of finding this same curved element in the somatic cells of the male, or else of not finding it at all since I have always suspected it of being compound in nature. If my mind were prejudiced, it was decidedly in favor of finding a

single, curved element in the male, and a pair of such elements in the female, instead of just the reverse—the actuality of which slowly forced its way upon me as I examined more and more preparations.

Where the chromosomes are sufficiently separated to make one reasonably sure that they were all present without overlapping, I find that I have recorded eighteen, rarely more, as the prevailing number. In other cases, the number visible is set down as 16, or in some few cases fifteen and seventeen; but in the latter cases since all of the smaller ones can not be identified, I have felt that it is a reasonable presumption to suppose that eighteen were there but that one or more of the smaller ones have adhered to or been obscured by some of the others. Thus my counts as recorded in one set of observations run as follows: 123 cells with eighteen chromosomes; 73 cells with sixteen chromosomes visible; 25 with seventeen chromosomes in evidence; 23 cells with fifteen chromosomes in view. Counts can only be made from polar views of equatorial plate stages. Attempts to determine numbers from side views were all futile. To add to the confusion in side views the chromosomes do not all divide at the same time so that some of the daughter chromosomes are well along toward the poles while others are still at or near the equator.

Sometimes instead of the expected number of conventional chromosomes, fewer of the latter are in evidence and the field of the mitotic figure, as seen in polar view, is peppered full of much smaller, deeply staining bodies which appear to be chromatin particles. If one counted each of these a chromosome then the number of chromosomes would sometimes total as many as forty or fifty. I am still at a loss to know whether these are particles of linin or mitochondrial material which take the same dyes the chromosomes do, or whether they are fragmented chromosomes. In favor of the latter view is the fact that occasionally, in lateral views, division figures are to be seen in which these particles lie in the equatorial plate as if ready for individual division.

The particular interest regarding the chromosomes of the spermatogonia centers about the two curved elements which were so frequently in evidence (photos 1–3, Figs. 70–99). They could frequently be picked out even when the other chromosomes

were so massed as to prevent an accurate enumeration beyond observing that other curved ones comparable to the pair in question did not occur. In general, the other chromosomes were smaller and usually appeared as straight, very short or moderately long rods (photo 1, Fig. 72). In some instances a number of chromosomes appeared curved and a particular pair could not be picked out with certainty though such cases were relatively rare (not over 15 per cent. of the cases) when compared with the number in which the typical pair were observable. Many of the cases which at first seemed to be exceptions were, upon careful scrutiny, resolvable into instances in which the ends of two chromosomes swung together, forming a V which at first sight had been mistaken for a single curved element. Fig. 76 shows such an instance; the pair at the right might easily be mistaken for a single chromosome. This mistake is particularly easy to make since the real curved elements frequently show an abrupt bend rather than a long even curve. It is also an error easily fallen into with material stained in iron-hæmatoxylin unless the preparation is strongly decolorized.

As is likely to be true in much cytological material in general, many cells, although in stages of division, were so affected by the reagents or lay in such a position as to render them worthless for accurate observation. These have necessarily been disregarded as it was wholly impossible to affirm that they either bore out or negated the observations made upon more favorable material. Where figures or photos have been made from sections showing only a part of a cell or a part of the chromosomes, as was frequently the case, the preceding and following sections have been carefully inspected to insure as far as possible that the condition intended to be conveyed by the figure is not a misleading one.

Confusion is most likely to arise in late prophase, when the chromosomes have arrived at their rod-like form but have not yet settled down to their final size and position before division. Then, all may show some degree of curvature and one cannot identify with certainty the special pair.

While many observations were made upon the dividing cells of the embryonic testis, as a matter of fact the most satisfactory

details were to be seen in the dividing cells of the nephridial tubules. The chromosomes of this tissue tend less to stick together than do those of the testis and they also stain more sharply. One obvious reason for the greater clearness of equatorial plate stages in such nephridial cells is due to the fact that in dividing to lengthen the tubules, the chromosomes lie at the equator of a spindle which has its longitudinal axis across the shorter diameter of the cell (Fig. 104). This shortening of the spindle produces a proportionally greater equatorial spread with the result that the chromosomes are spaced further apart. The legends accompanying the respective photos and figures state whether the picture in question is of a somatic or of a germ cell.

Photos 1 to 3 and Figs. 70 to 99 show representative conditions in various somatic and spermatogonial cells. Most of them speak for themselves. In such figures as 86, 87 and 98 the pairs of special elements appear as of inordinate size. This is due in part to their actually greater size but also to the fact that the ends of most of the other chromosomes have been cut away in the preceding and following sections.

Photo 3 is of a spermatogonium of an adult cock. While the ordinary chromosomes were clumped so that little detail could be determined beyond the fact that there were no long curved elements among them, the two special elements lay well to one side and were particularly easy to identify. As already mentioned the spermatogonia of adult fowls were much less satisfactory to study in detail than were those of embryonic chicks. However, various other spermatogonia in adults, showing the two curved chromosomes, were found as were also a number in the guinea-fowl, and hosts of them were discovered with two special projecting elements which one suspects of being the same curved elements as are visible in spermatogonia and somatic cells when conditions can be clearly seen, but concerning which one is not absolutely sure. In any event such conditions do not negate the evidence found in the more favorable cells.

The relative positions of the two elements usually in evidence were, for example, approximately those indicated in such cells as photos 1 and 2 and Figs. 71, 72 and 76. That is, when seen in

polar view, they lay most commonly with their ends toward the periphery of the equatorial plate and their plane of curvature at right angles to the chief axis of the spindle, and frequently they were relatively near together, being separated by only some two to four small chromosomes. Sometimes, however, the two curved elements lay at opposite sides of the spindle as in Fig. 86. Rarely the position of the two bodies on the spindle was such that one had its plane of curvature parallel to the long axis of the spindle.

The Chromosomes of the Female.

Before continuing with the later phases of spermatogenesis it will be well to consider the condition of the chromosomes in the early germ and tissue cells of the female chick. Photos 4 to 7 and Figs. 100 to 117 show representative stages of the chromosomes in the primordial oögonial divisions and in the divisions of somatic cells as seen in the nephridial tubules. The latter, as in the male, were often the more favorable for study. The divisions of the primordial oögonia, indeed, were considerably more difficult to decipher than those of the spermatogonia because for some reason, possibly correlated with the larger size of the cell, the chromosomes were frequently longer and more thread-like and consequently more likely to interlace and otherwise alter in fixation. Although in the ten-day chick whole nests of oögonia would be found in various stages of division it was rare to find stages that one could make heads or tails of when it came to studying individual chromosomes. It was hopeless to try to do anything with the late prophase stages because the chromosomes even up to entering the equatorial plate remained decidedly elongated and nearly all of them showed more or less curvature. However, not a few dividing oögonia were found in such condition as to show that a single characteristic element was commonly present. While several chromosomes in a given equatorial plate might show more or less curvature, this special element, commonly larger than the others, was usually discernible in such division figures as showed the chromosomes in any condition beyond that of a confused mass. In somatic cells, especially those of growing uriniferous tubules, conditions were considerably better. The individual chromo-

somes were not so long and the special, unpaired curved element was frequently in evidence. While in some cases I have recorded the occurrence of more than one curved element in individual cells, these additional curved chromosomes were usually smaller than the element in question and readily distinguished from it. In the first hundred polar views of dividing cells showing any understandable detail, recorded from one preparation of a ten-day female chick, for instance, I find that 43 have a single unmistakable large curved element, 20 have a long element which is probably the curved element with the curve turned directly away from or toward the observer, and therefore out of perspective, 27 have a number of the chromosomes curved so that it is impossible to pick out any special one as the particular element in question, and 10 do not show what could positively be identified as a special curved body, the doubt arising as to whether what appeared to be curved element was not two chromosomes with the ends overlapping.

Photos 4 to 7 and Figs. 100 to 117 show characteristic appearances of the cells of females as revealed by the photographic camera, or the camera lucida. For the details regarding each figure the reader is referred to the legend which accompanies it. Fig. 115 represents a type found occasionally in which while an actual curved element was not to be found, an individual chromosome much longer than its mates was to be seen and is probably to be interpreted as the special element in question. Not infrequently it held the hæmatoxylin stain much less tenaciously than did the ordinary chromosomes, becoming yellowish brown in color, while they remained black. Fig. 110 shows this lighter staining element as unmistakably curved. Photos 6 and 7 are side views of equatorial plate stages. Each shows a special elongated chromosome at one edge of the chromosomal plate. While these elements do not appear in the photograph to be curved as a matter of fact a slight shift of focus of the microscope reveals a decided curvature in each. Although in many cases the flat surface of the curved element was turned at right angles to the long axis of the spindle (Figs. 107, 110), not infrequently it lay parallel to the latter as in Fig. 108 or even at an acute angle.

Fig. 111 shows a late metaphase in which the special element is dividing lengthwise. Figs. 112, 114 and 117 illustrate various cases in which division is in progress or has occurred.

From time to time an especially bothersome element appeared in some cells which complicated the interpretation of conditions in the tissue of females. It took the form of an elongated rod which occurred along with the curved element. Like the curved element, it often was of lighter staining capacity and had the former not been distinctly visible as a separate body the second element might have been mistaken for it. Whether or not it consists of several of the ordinary chromosomes which have remained attached and are dividing as one body is not wholly clear. This seems to be the most plausible interpretation although it is not apparent why such a mass should stain less deeply. That such compounding does occur occasionally in the cells of various vertebrates, I am strongly inclined to believe, from the evidence I have seen of variations in the number and size of chromosomes in other avian and mammalian tissues. Apparently chromosomes in such cases represent congeries of units of a lesser order which may be done up in fewer and larger, or more numerous and smaller packets, contingent upon as yet unknown conditions of equilibria in the cells. This probability constantly hangs over the student of these forms as the chief one which is likely to vitiate his conclusions. All the corrective he has is to make great numbers of observations and base his conclusions upon the conditions which he finds strongly preponderant.

Figs. 101 and 102 are drawings made from dividing cells in a five-day chick. While sex cannot be determined from macroscopic evidence, or even microscopic examination of the indifferent gonads at this early date, from the fact that the cells each contain but a single large curved element the inference would be that the embryo bearing these cells is a female. In this connection, the writer was greatly interested in Fig. 2 of a recent paper by Swift ('15). The figure shows a section through the indifferent gonad of a four-day chick embryo and includes a division figure of a primordial germ-cell. Although Swift's study was not on chromosomes and he does not mention the

condition of the chromosomes of this particular cell, he has unwittingly, and therefore without even subconscious bias, given us what appears to be a beautiful example of a single curved or U-shaped chromosome among a number of straight rod-like ones. Unfortunately the cell is so cut that a few of the chromosomes lie in another section so that the evidence is not absolutely convincing although since nearly all of the chromosomes are shown, it appears to be decidedly corroborative of my own findings.

Primary Spermatocytes.

The later spermatogonia, in the resting condition, very commonly show two chromatin nucleolar-like bodies which, judging from the fact that one or both often display a decided curvature, are possibly to be identified with the two curved elements of the division stage. In some cases the resemblance is decidedly clear, in others less so. Sometimes the bodies appear to be spherical or oblong rather than curved, but this appearance is due in some instances at least to the position they occupy with reference to the observer. Fig. 118 shows a group of such spermatogonial nuclei as seen in a section of the testis. Parts of the nucleolar-like bodies have been cut away in some but in the nucleus below and to the left, one element is present in its entirety and so oriented as to show its curvature. Later when the growth period begins these bodies tend to fade out although in such stages as Fig. 119 they are still visible. The disappearance seems to be in the main a loss of staining capacity (Fig. 121) rather than an actual dissolution.

During the period of growth and development in which the products of the spermatogonial divisions become primary spermatocytes although important activities are obviously in progress in the nucleus, I have been unable to determine sufficient constancy in the details on which to base an adequate conclusion regarding such important processes as synapsis. There is the usual increase in nuclear size and with it characteristic appearances at certain stages. For example, there is an apparently early stage in which the nucleus seems to have the chromatin material scattered through it in the form of a fine dust, with occasional fine strands of linin and small fragments of chromatin

in evidence (Figs. 119, 120). Then follow stages in which the stainable content seems confined to a very fine spireme with the chromatin practically all concentrated in the filaments (Fig. 121). In especially favorable preparations it can be seen to be strung along as a string of very small round chromomeres. This stage is evidently the leptotene stage of modern literature. Whether the threads are separate and as numerous as the diploid chromosomes or whether they constitute a continuous or a discontinuous spireme could not be determined. It can only be averred that the strands are much finer, and more numerous than those which exist just prior to the appearance of the individual chromosomes. Any number of instances of two of the fine filaments lying parallel one to another could be cited, but whether such threads constitute true pairs in process of parasynapsis or whether the condition is purely incidental could not be determined. Regarding the question of parasynapsis I can only affirm that the evidence is certainly not against such an interpretation and as far as it indicates anything it rather points toward parasynapsis than otherwise.

Consequent upon the leptotene stages come the contraction phase or synizesis in which the filaments condense into what appears to be an indiscriminate tangle (Fig. 122), and then slowly follows a second extension of the chromatic filaments throughout the nuclear area until the nucleus is occupied once more by a spireme, this time of fewer and coarser filaments (Figs. 123, 124). In Fig. 123, two elongated, nucleolar-like elements are visible.

The spireme in its various phases is a stage of considerable duration if one may infer from its universal presence in almost any section taken from an active testis. When it once starts to condense into the individual chromosomes the operation, judging by the scarcity of stages to be found, is a comparatively rapid one. Just how the bivalent chromosomes form from the spireme I have not been able to discover despite much time spent in the attempt to do so. One finds a relative abundance of metaphase stages but in every case the chromosomes, when identifiable, are well established, relatively compact bodies. The most diligent search has failed to reveal the accessory chromosome or chromo-

somes conspicuously laid down in advance as nucleolar-like bodies, after the manner recorded as so characteristic in insects. Spiremes and synizetic stages may be found occasionally which show one or more chromatin condensations which might be interpreted as nucleoli but they rarely show anything in size or shape which could lead one to identify them with the curved elements of the spermatogonia or of the larger special element found later in the spermatocytes.

Figs. 125 to 132, in which all details have been depicted as accurately as possible, show representative views of an interesting condition which exists just prior to the formation of the chromosomes in the primary spermatocytes. The spireme of such a stage as that shown in Fig. 124 seems gradually (Fig. 125) to break up into a series of characteristic smaller and larger chain-like groups. In some of these it is difficult to decide whether each formation consists of a series of transparent, bead-like bodies encased in a thin shell of deeply staining material, or whether it arises through the twisting of two filaments one about the other. The latter is certainly the condition in some cases, particularly of the smaller elements where the free ends of the threads are distinctly visible, as for instance in Fig. 130, and I am inclined to think that it also prevails in the other instances. The formations thus established condense gradually into bodies of smaller size, certain ones of which, at least, take on the appearance that is so characteristic of some types of tetrads (Fig. 132). Even in metaphase, when the chromosomes of the primary spermatocytes are well established, a tetrad-like condition of individual chromosomes may occasionally be detected in strongly decolorized preparations. In some instances, indeed, one member of such four-groups apparently becomes displaced and divides as a smaller, independent member, thus confusing the chromosome count. Occasionally this seems to occur in more than one member of a given set of chromosomes so that two or more of such quarter-sized individuals may appear at the formation of the equatorial plate stage.

As noted in my earlier paper ('09*b*) there are frequent nuclear divisions without a corresponding division of the surrounding cytoplasmic mass. Commonly from two to four large primary

spermatocyte nuclei may be seen in process of growth or of division in a common mass of cytoplasm which shows no indication of being divided into separate cells. The condition often persists through the following division stages with the result that from eight to twelve spermatid nuclei may be found in one syncytial mass. More frequently, however, what appears to be a fragmentation of the cytoplasm without the appearance of definite walls, into clumps containing fewer nuclei occurs. Looked at from the standpoint of the relative rôles of nucleus and cytoplasm in heredity, this establishment of specific nuclei in a more general matrix of cytoplasm might be regarded perhaps as indicating the more individual nature of the former and more generalized constitution of the latter. On the other hand, inasmuch as but little cytoplasm enters into the makeup of the ultimate spermatozoön the condition may not be of as much significance as one is at first thought tempted to attribute to it.

Notwithstanding the scarcity of intelligible prophase stages in the primary spermatocytes, at metakinesis, there is to be found in abundance a characteristic chromatic element of constant shape and size which behaves like the typical X-element of insects. Whatever the theoretical interpretation may be, the presence of this element can be abundantly demonstrated. It not infrequently comes to be at or near one pole of the spindle while the ordinary bivalent chromosomes are still in the equatorial plate stage (photos 8–13; Figs. 134, 137, 145, 146, 149). At a slightly earlier period it is nearer the equatorial plate—commonly just above or below (Photos 15, 20, 22; Figs. 135, 138, 139, 159), but very frequently also at one edge (Figs. 148, 154–158). Unless preparations are very strongly destained it is likely to escape detection in such positions as the last, since in heavily stained preparations the whole chromatic figure becomes a blurred mass. In fifteen cases I find I have recorded curved elements at each pole of the same spindle, the presumption being in such instances that the original special chromosome has divided as do the ordinary bivalent ones of the spermatocyte.

Boring and Pearl ('14) have published a paper on some phases of the spermatogenesis of the domestic chicken. They find

little or no evidence of such an element as I have described beyond what might charitably be regarded as a mere accident. Although they say that "it is impossible to count chromosomes accurately in this material," and that to attempt to work out a continuous detailed history of spermatogenesis in the Barred Plymouth Rock "would require a large amount of imagination" they have not hesitated to label a number of their mass-effects as containing "no possible X." While they assert that some 15 per cent. of their preparations show a possible X-like element, 85 per cent. do not. It is only a fair question to ask if this 85 per cent. shows anything else, and if the pictures they give are a fair sample of the whole, I think any unbiased observer will have to admit that they do not.

I agree with them in finding that it is very difficult to get a satisfactory count of chromosomes except in the most favorable material. But even without an absolutely accurate count it can be determined in many preparations of chicken testis that there is one peculiar curved or bean-shaped element present unlike the others which are round or oblong, and above all, an element that behaves in a characteristic way. When such observations can be strengthened by further unequivocal cases where a count is obtainable, then it seems to me that legitimate conclusions can be drawn regarding what is the usual occurrence in the material in question. The evidence as seen under the microscope undoubtedly shows that there are sometimes such fusions of the ordinary chromosomes as to reduce the count—and this is, as I have already maintained, a constant factor in secondary spermatocytes—but this by no means invalidates the evidence regarding a special X-like element, inasmuch as fused ordinary chromosomes would not frequently be mistaken for it.

In doing cytological work I have always accepted as a working maxim that preparations which show nothing definite must be ignored and only those taken into account which have sufficient distinctness to be capable of a reasonable interpretation. Almost any cytological preparation I have ever seen—and I have examined preparations of some of the best technicians in America and Europe—will show a number of dividing cells, let us say, which can not be said to give evidence of anything much beyond

the fact that the cell is in process of division. To determine detail regarding a particular chromosome, one naturally has to ignore such preparations and choose only those cells which either show evidence for or against the point at issue. There is always a residue of cells which must fall in the neutral zone of non-significance, and this residue of course becomes increasingly great when one is dealing with inherently unfavorable material, as the germ-cells of the rooster undeniably are. Even in as classical and clear cut an object as *Ascaris* with its small number of chromosomes, I find that a large percentage of the cells showing chromosomes give no clear cut evidence of the conditions which we universally teach as characteristic of *Ascaris*. On the other hand they do not negate these teachings.

Regarding the X-like element of the male fowl I found that it exists in great abundance. Up to date I have in my notes 963 unequivocal cases recorded in primary spermatocytes, to say nothing of many other cases I have seen but not specifically noted down. In the field under the microscope from which such photos as 9 and 34 have been taken, under slightly lower power some 3 to 5 other division figures each showing such a curved element may be seen. The element in question when seen from the side is always in the form of a curved rod, usually thick and plump looking, though occasionally more slender and proportionately longer. Photo 8 is a good clear-cut example of how it appears in a favorable preparation. Inasmuch as it may lie in almost any conceivable position with reference to the pole of the spindle, or the point of view of the observer, obviously the majority of views of it will be at some other angle than that depicted in photo 8 and correspondingly difficult to represent by photography. Indeed, for every one to be found in a suitable position to photograph, many could be positively identified as the same element, but lying in such a plane that some shift of the fine adjustment of the microscope was necessary to see the whole chromosome. Often tangentially lying elements or those with the curved surface turned directly toward the observer require careful focusing to determine the exact shape and dimensions of the object.

Photos 12, 14, 23 and 27, when examined under the microscope

where slight shifts of focus are possible, are just as clear cut cases as photo 8, although because of foreshortening of the object as seen in one plane, not one of them gives as convincing a photograph.

Figs. 152–158 and photos 30–32 are polar views showing the X-like body lying at the edge of the equatorial plate. If such a cell were being viewed from the side instead of the pole it is obvious that the special element would in all likelihood be indetectable and such a view would probably, by one who desired to make a case against the existence of such a body, be recorded as evidence against it. Such side views must of course occur, but in even many of these a careful scrutiny of the mitotic figure shows that it is asymmetrical, extending out further on one side from the spindle than on the other. In such instances it is as legitimate to infer that one has in the chromatic band before him a special element related to the other chromosomes as in Fig. 157, as it is to consider it in any other way. Or in any event it can not be legitimately recorded as evidence against the existence of such a body. It is obvious further that if such an element as shown in Fig. 157 lay directly back of or in front of the other chromosomes as viewed by the observer, instead of at the side, the equatorial plate would then appear symmetrical and such a stage, though having the element in question, would be likely to be recorded as without it.

Photo 33 shows a telophase of a dividing primary spermatocyte in which the undivided X-element lies close to one set of the divided chromosomes after the latter have migrated to their respective poles. The opposite pole has no such body. Photos 34 and 35 show somewhat similar conditions, only in these cases matters are complicated by the beginning of the secondary fusion of chromosomes which is so characteristic a procedure preliminary to the next division. In such cases, of which a number have been observed, the X-like element is apparently tied to the rest of the adjacent chromatin mass by two heavy linin fibers (Figs. 151, 164, photo 34). It is possible that this is determined by the fact that the curved chromosome is in reality probably double in nature. Figs. 150, 151 and 164 are camera-lucida drawings of such conditions showing details that could not be revealed by photography.

In my earlier paper ('09*b*), based upon a much more meager series of preparations and on the whole considerably less satisfactory from the standpoint of technique, I made mention of a third chromosome which was at times associated with the curved one. A more extensive survey of material shows this to be of much less frequent occurrence than I then thought it to be, and probably of no special significance. Such a smaller chromosome may infrequently be seen either toward one pole along with the X-like body or alone, or even toward the opposite pole. So far as I can analyze the condition it is merely one division product of one of the smaller chromosomes of the equatorial plate which, following a precocious division, has passed on in advance of the other chromosomes toward one pole. My earlier drawings also give the impression of considerable irregularity in the outline of the chromosome there designated as the odd. While such irregularities may be found I must conclude from my later material that the appearance is due in the main to unsatisfactory fixation as my recent, better preparations all show what, when one considers the difficulty of the material to be handled, is a surprising uniformity in the appearance of this body. Photo 8 illustrates what may be regarded as the type. Indeed, in my study of this element, as I came to examine by the hundreds division figure after division figure it became impressed upon me that this was in reality the one most constant identifiable element in the whole phase of spermatogenesis.

If this X-like body is merely accidental, then it is one of the most astonishingly consistent accidents I have ever encountered. It has a relatively constant size and shape, it ordinarily is not likely to be confused in the least with other elements present in the spermatocyte, and it is an accident which has the very same appearance in the testicular cells of Langshan, Plymouth Rock and Rhode Island Red fowls. Furthermore, a similar accident occurs in the guinea-fowl, only there it is consistently comma-shaped (Fig. 160) instead of being a curved element of uniform thickness. Moreover, in the guinea-chicken hybrid a similar element resembling more that of the guinea parent can be identified, and lastly, on the accident theory, the crowning wonder is that in all of these forms, the element in question

should imitate so consistently the behavior of an X-element. It seems to me that one must be credulous indeed to catalog it all as accidental.

When I take into account all of the evidence which I have been able to accumulate through some ten years of study on this problem, I feel more firmly convinced than ever that in this distinctive curved chromosome we are dealing with a body comparable to the so-called X-element of other forms. Certainly the evidence of this, as regards numbers of X-like chromosomes actually seen is much stronger than that which has been accepted unquestioned for certain of the less typical cases in invertebrates. However, in view of the condition which I have found existing in certain somatic and early germ-cells of the male where in a large number of instances two special curved chromosomes occur, I am strongly of the opinion that the large curved element of the primary spermatocyte is in reality these two earlier elements fused into one. It is just about the size that two such elements would have when fused; moreover, no further trace of the latter is discernible after the spermatogonial stages are passed. Then again, such a doubling would account for the relatively larger size of the curved element in comparison to the other chromosomes of the primary spermatocyte. My interpretation of the condition then, insofar as I can analyze the probabilities of the case, is that the two special curved elements of the spermatogonia fuse in the primary spermatocyte and act as a single element, typically passing undivided to one pole of the mitotic spindle and thus producing a dimorphic condition of the ensuing spermatocytes of the second order, half of which will contain the X-like element, half be without it.

Secondary Spermatocytes.

The secondary spermatocytes are of approximately the same size as the original spermatogonia. Although resting nuclei are not infrequently to be found, in many cases the chromosomes of the primary spermatocyte telophase rearrange directly to form the metaphase of the secondary spermatocyte. In so doing there is a marked tendency for the chromosomes to fuse by twos so that instead of the expected sets of eight and nine re-

spectively received from the primary spermatocyte, a smaller number, usually four (Figs. 175–183, photos 36, 37) or five (Figs. 161–163), appear at the equator of the new spindle. The groups of four I interpret as characterizing those cells which received only the eight ordinary chromosomes at the preceding division, the groups of five, those which received the extra, curved element in addition to the eight ordinary ones. This view is strengthened by the fact that in the groups of five, the fifth element (Fig. 163) may often be seen to resemble the original special element of the primary spermatocyte. Furthermore, at the time of division this element lags behind like a typical X-element and often does not divide until the other chromosomes are well on their way toward the poles of the spindle (Figs. 168, 172, 174, photo 63).

While my best preparations show groups of four and of five respectively to be by far the prevailing type, it is not unusual to find other cases in which the number may be six, or seven—any numerical combination, in fact, that can be made by the union in twos of one or more pairs of chromosomes out of a total of eight. The explanation seems to be that while pairing of the ordinary chromosomes is the rule, this union is sometimes incomplete or does not occur between certain individuals. In both the four and five groups one of the chromosomes is smaller than the others (Fig. 177) and where only one pair of the autosomes remains unpaired in the secondary spermatocyte, it seems most frequently to be the two components of this smaller bivalent one (Figs. 184 and 185). This gives five chromosomes without the X-like one in what typically is a four-group, and six chromosomes in what otherwise would have been a five-group where the curved element is present. In early metaphase of the secondary spermatocytes a bipartite condition of the chromosomes preparing to divide not infrequently reveals their double nature (Fig. 180). Moreover, the chromosomes may tend to resolve into their original univalent condition as they progress toward the poles presumably after having divided as a four or five group (Figs. 168, 172). The occasional finding of groups of eight or nine univalent chromosomes at one pole or the other in the telophase of such a division indicates that the division is in no sense a reduction division as was probably the preceding one. Fig. 164

and photo 34 show cases in which the chromosomes which have passed to the poles of the spindle in the primary division are in process of rearrangement for the secondary division, the X-like body remains with one group only. The autosomes have fused or are fusing in pairs.

In a very few instances spindles bearing the same number of chromosomes as occur in the primary spermatocytes were found. The chromosomes were relatively small as if of the univalent type. These are possibly to be interpreted as secondary spermatocytes in which the usual fusion has not occurred, but on the other hand the evidence is not clear that they are not primary spermatocytes in which the fixing agent has caused an unusual shrinkage. In any event, supposing these are in reality secondary spermatocytes, out of thousands of secondary spermatocytes examined I have found only fifteen cases of this kind. All the others, even where the exact number of chromosomes could not be determined, showed a chromosome number that was certainly less than that of the primary spermatocyte.

There has been a disposition on the part of some to question the existence of this second diminution in chromosome number. In my own work I first came across it in studying the spermatogenesis of doves and pigeons (Guyer, '00) in which forms its existence has also been confirmed by the more recent work of Geoffroy Smith ('12). Later I described it again in the guinea-fowl ('09*a*) and the chicken ('09*b*). A similar occurrence has been recorded for several mammals (*e. g.*, Jordan, '11, Wodsedalek '13). In any event, the fact remains that four-groups and five-groups are present in abundance in the testis of the common fowl. They are so obvious indeed at every turn in a good preparation that it has been a long standing puzzle to me why there has been any difficulty on the part of others in demonstrating them. They have become an object of routine demonstration in my own cytological laboratory which even beginners in the course have little difficulty in finding.

Photos 36–54 show various groups of four. Photos 47–52 represent anaphases where even in a photograph, with its limitations as to planes, four chromosomes are revealed at one or both poles. In photos 51 and 52, to show detail at one end the other

has had to be thrown out of focus but the four chromosomes can be demonstrated there just as clearly. Figs. 186–188 show more clearly the conditions which exist in these four-groups inasmuch as some depth of focus has been added. It is obvious that, as in photos 42–46, where the chromosomes are arranged around the equator of the spindle and the latter is seen from the side, only three of the chromosomes can be photographed as the fourth lies behind the middle one and in a different plane. Such photos as 37 and 41 of polar views reveal the true condition of affairs. Figs. 181–183 show the real constitution of such groups when seen from the side under the microscope where the focus can be shifted.

Photos 55–64 and Figs. 165–174 show views of five-groups. Photo 61 and Figs. 169–171 represent such a group in anaphase of division. One end is in better focus than the other in the photograph; the figure (Fig. 169) reveals the true condition which is as clear cut as if stamped with a die when examined under the microscope. Photos 63 and 64 are of five-groups in which, although the regular chromosomes have divided the X-like one has lagged at the equator of the spindle and is just in process of division. Figs. 167, 168, 172–174 show camera lucida drawings of other somewhat similar stages.

Spermatids.

Inasmuch as the secondary spermatocytes were dimorphic the condition is maintained in the spermatids, of which, therefore, there are typically two classes; namely, those which receive four chromosomes (probably eight univalent ones) and those which receive five chromosomes (probably nine univalent). In most cases, at the conclusion of the last division the chromosomes mass more or less and become a center around which the new nuclear membrane appears.

Although resting spermatids with reticular nuclei are to be seen, frequently the spermatids seem to proceed to spermiogenesis without a vegetative or resting stage; that is, the chromosomes seem to mass for the transformation without passing back into the diffuse stage.

It should be mentioned at this point that some of the spermatid

nuclei seem to proceed to further division. This anomalous division, in so far as I have been able to follow it, seems to be confined to the spermatids which have received the four chromosomes. Photo 69 shows in the upper field a telophase of a secondary spermatocyte which is dividing as a four-chromosomed cell to form two spermatids which will obviously contain four chromosomes each. Below it, however, is such a division of a four-chromosomed spermatid as I have just mentioned. The size relations of the two are obvious. Figs. 193 and 201 show similar conditions. I am inclined to believe that even further divisions of such four-groups occur inasmuch as it is not uncommon to find still smaller cells with four small chromosomes in process of division. Photos 65, 66 and Figs. 196 and 197 represent such a type. They may become so small as to make it difficult to decide whether one is dealing with 4 small chromosomes or a quadripartite chromosome. On the other hand, one may from time to time encounter division figures in which, instead of four chromosomes, only two appear at the equator of the spindle (Fig. 198, photo 68) suggesting that the fours have again paired.

Such unaccountable behavior on the part of the many four-chromosomed cells suggests that these cells have run riot, as it were, and are to be looked upon as degenerating. This condition, together with the fact that there are many cells among the spermatids which look as if they were not normal (Fig. 204) makes it appear probable that the one class of spermatids does not transform into adult spermatozoa. Moreover, careful measurements of spermatozoa from the vas deferens fail to reveal more than one type.

Fig. 204 represents forms which occur very commonly among the spermatids. Instead of an elongation of the nucleus and a rearrangement of the chromatic bodies into the form characteristic of what may be interpreted as the normal course of development (Figs. 200, 203), the chromatin compacts into a single dense round or very irregular mass. Occasionally what looks like an incipient axial filament appears but does not develop far. At least, I would so interpret the many instances which occur as represented in Fig. 204. Not infrequently also

spermatids are to be found which are indefinite in nuclear make-up and seemingly impaired in some way.

It cannot be positively affirmed, of course, that such cells as are represented in Fig. 204 are the product of the four-chromosomed class instead of the other. Such an interpretation is merely an implication which grows out of what appears to be other aberrancies in the behavior of the four-chromosomed class.

While at first glance, this view that one entire class of spermatids degenerates without becoming functional may seem improbable to some, it should be borne in mind that such an occurrence is by no means unique in spermatogenesis. In both phylloxerans and aphids, for example, just such an aborting of one of the two classes of sperm-forming cells occurs, to say nothing of the even more remarkable conditions which obtain in bees and in certain hermaphroditic forms such as *Rhabditis*.

Figs. 200 and 203 represent stages in what I interpret as the normal sequence of transformation. In such cases, by the time the axial filament first appears the nucleus has already begun to elongate slightly. Typically the chromosomes from the last division seem to arrange themselves into more or less of a closed ring around which the nuclear membrane forms. They then tend to concentrate gradually toward one side of the nucleus along the periphery until they form more or less of a crescent which thickens and shortens as the process continues (Fig. 200). The nuclear membrane, along the margin free from chromatin, fades from view as the transformation progresses, leaving finally an elongate chromatin mass rounded at one end and more sharply pointed at the other. The pointed end is seen to be provided with a definite head spine and the blunter end to be in juxtaposition to the axial filament. The latter seems to develop in the typical way from the divided centrosomes, one of which has become knoblike and the other a ring.

Instead of following what appears to be the simpler course of development as depicted in Fig. 200, the nucleus seems frequently to transform into the spermatozoön head by a process of uncoiling, representative stages of which are shown in Fig. 203. As is the case in other various types of spermiogenesis, fragments of cytoplasm appear not infrequently to be cut off from the

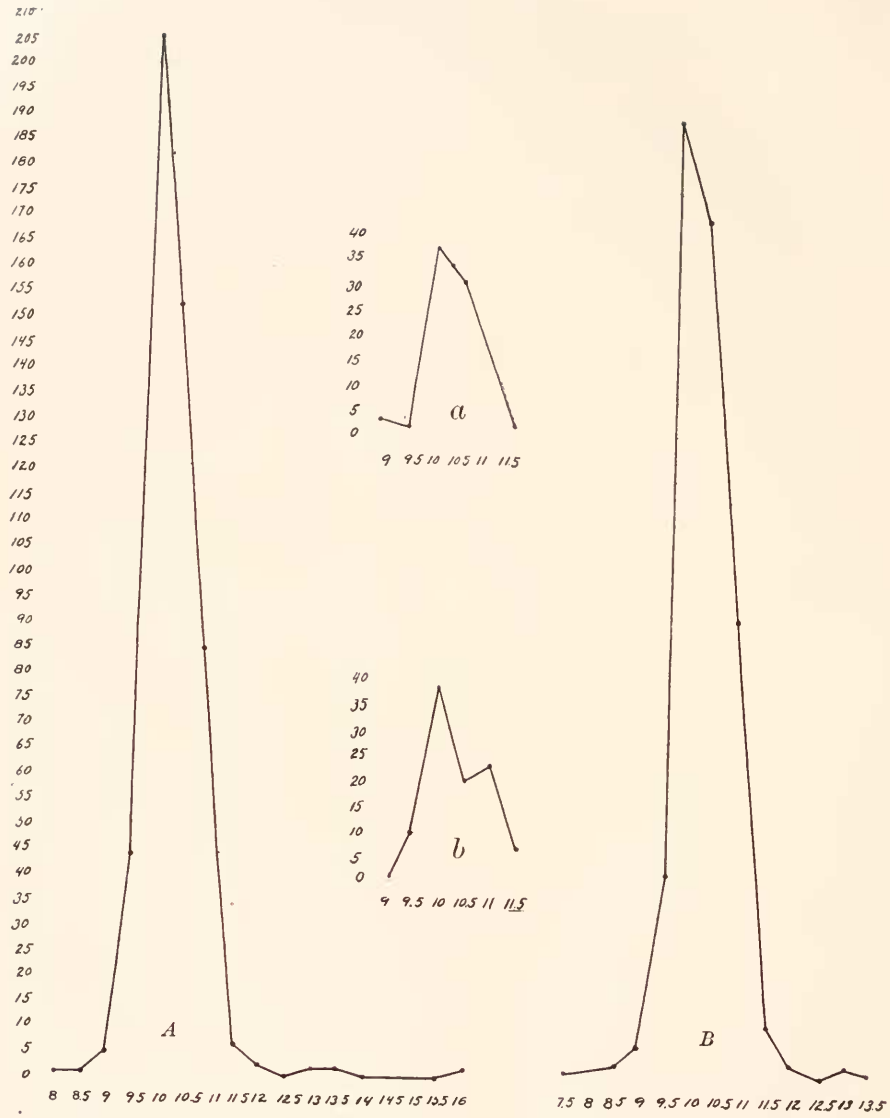
cytoplasm of the transforming spermatid and left behind. It is not easy actually to establish this, but such would be the inference based upon the many non-nucleated bits of cytoplasm scattered among the spermatids, together with the fact that lobes of cytoplasm may be seen projecting from many of the transforming cells. On the other hand it is possible that these bits are cytoplasmic fragments from degenerating spermatids.

Spermatozoa.

In my earlier paper I came to the conclusion that there were two classes of spermatozoa. My present work shows that I was probably in error on this point. My earlier work was based on measurements of the heads of 100 spermatozoa as they existed in smears of the testicular material itself. While no curve was plotted my older notes show that I obtained two means standing in about the relative ratios of 8 to 10. What would have made the $8\frac{1}{2}$, 9, and $9\frac{1}{2}$ groups, however, would afford but slight depression in a bi-modal curve. Furthermore, when later I came to measuring spermatozoa from the vas deferens, as I should have done for the earlier work, I found that the spermatozoa taken from testicular smears show a larger size and a far greater range of fluctuation in length of head than do those from the vas deferens. This means that doubtless many of those I measured in my earlier work had not yet completed their transformation and settled down to their final size.

The present measurements were made in four series based upon the length of head of spermatozoa taken from the vas deferens of two Plymouth Rock fowls. Two of the series, one from each fowl, including measurements of 500 and 515 spermatozoa respectively, I myself made. The other two, each consisting of measurements of 100 spermatozoa, were made as a control by an instructor (Dr. Elizabeth A. Smith), who is accustomed to doing cytological work but who was wholly in the dark as to what result had been obtained in my own measurements. The instructor worked with material from the same fowls but on different slides from the ones I used. The details are shown in Text-figure 2.

It is by no means an easy task to obtain such measurements



TEXT-FIG. 2. *A*, frequency distribution of head-lengths of 500 spermatozoa from a Plymouth Rock fowl.

Scale value	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5	14	14.5	15	15.5	16
Frequency	1	1	5	42	206	151	83	6	2	0	1	1	0	0	0	0	1

a, frequency distribution of head-lengths of 100 spermatozoa from the same fowl in a different preparation and by a different observer.

Scale value	9	9.5	10	10.5	11	11.5
Frequency	3	2	37	31	25	2

with the necessary degree of accuracy. It is not always easy to determine the exact base or the exact apex of the head. The most vexatious fact was that the long heads of nearly all of the spermatozoa were slightly curved and it required much searching to find ones sufficiently straight to measure accurately. Other likely sources of error were guarded against as carefully as possible. As nearly as could be done exactly the same fixation and degree of staining was secured in each slide. Care was taken to see that the film of seminal fluid was spread on in approximately the same degree of thinness and that only such parts of the preparation were studied as appeared to be wholly free from stretched or distorted spermatozoa. To eliminate the personal equation, as already related, another person was obtained to do two series of counts. Still other sources of error such as might arise through foreshortening of the object to be measured, or fatigue on the part of the observer, were guarded against.

A Leitz Stufen-Mikrometer in a No. 2 eye-piece was employed and the readings were based on measurements down to the mid-field between the individual lines. The lines, as is usual in micrometers, were laid off in tens. Inasmuch as relative sizes were all that was desired the figures accompanying the various curves represent merely divisions of the ocular micrometer. It is evident (Text-fig. 2) that three of the curves show no trace of bi-modality. A slight trace appears in *b*, but so slight as to be negligible since the curve is based on measurements of only one hundred spermatozoa and the actual differences between the 10.5 group and the 11 group is only three individuals, there being 20 spermatozoa which measured 10.5 and 23 which measured 11. Moreover *B* is a unimodal curve based on measurements of 515 spermatozoa from the same individual as that from which those measured in *b* were taken. With the differential X-like

B, frequency distribution of head-lengths of 515 spermatozoa from a second Plymouth Rock fowl.

Scale value	7.5	8	8.5	9	9.5	10	10.5	11	11.5	12	12.5	13	13.5
Frequency	1	2	3	7	40	188	169	90	10	2	0	2	1

b, frequency distribution of head-lengths of 100 spermatozoa from the same fowl as in *B*, in a different preparation and by different observer.

Scale value	9	9.5	10	10.5	11	11.5
Frequency	1	11	38	20	23	7

element as large as it is, if one half of the spermatozoa possessed it and one half did not, one would certainly expect to obtain a fairly well marked bi-modal curve in the measurements of such spermatozoa. The presumption is therefore that only a single class of spermatozoa are represented. The probability that this class is the one developed from the five-chromosomed spermatids has already been indicated. While one wishes that the evidence might be more decisive on this point, no more significant facts seem forthcoming under present conditions of technique. What evidence there is points to the interpretation I have given and I have found no facts which indicate the contrary.

Conclusions.

Many as are the pitfalls and unsatisfactory as are parts of the evidence, I feel that I have examined a large enough number of cases and have studied a sufficient number of interpretable stages in chromosome behavior to proclaim the foregoing account as substantially the ordinary course of spermatogenesis in the common fowl. I feel that the chief hiatus centers about the fate of the one class of spermatids; that is, as to whether the degeneration evinced is confined to the four-chromosomed class, whether this class never forms spermatozoa, and whether all cases I have regarded as abnormal are really conditions of degeneration. But in the light of the fact that as a result of the transformations of spermatids only one class of spermatozoa arises I feel that only one class of spermatids completed the course of final transformation.

To those who have followed the recent literature on sex-linkage in fowls the significance of the conclusions arrived at in this paper is obvious. The facts of such linkage have been reviewed so often in recent papers and books (*e. g.*, cf. Morgan, "Heredity and Sex," 1913) that it is needless to lengthen my paper by restating them here. It is sufficient to mention that certain characteristics such as color pattern are inherited in a manner to indicate that in fowls the female is heterozygous, the male homozygous, for sex and sex-linked factors. My earlier study in which the X-like chromosome therein described was regarded as an ordinary X-element and therefore presumed to exist as

one rather than a pair of elements in the male somatic cells made the cytological evidence, if we were to continue to regard this accessory element as specifically concerned with the sex-linked characters, apparently stand at variance with the facts established by breeders. The evidence as presented in this paper, if I have correctly interpreted it, does away with this difficulty, since the female soma as seen in chick embryos is heterozygous and the male soma is homozygous for a special curved element which to my mind fulfills the requirements for being regarded as a bona fida X-element.

Summary.

1. My later studies confirm my earlier ones as regards the finding of a large curved chromosome in primary spermatocytes, comparable to the so-called sex-chromosome of other forms.

2. The presence of this element is recorded in 963 primary spermatocytes which were sufficiently well prepared to show interpretable detail. It has been observed in many others. It probably exists in all although often obscured by the other chromosomes which tend to stick together.

3. It is, for variously fixed material, surprisingly constant in shape and size, in Langshan, Plymouth Rock and Rhode Island Red fowls.

4. A similarly constant element, differing in form from that of the common fowl, is found in the guinea and in the guinea-chicken hybrid.

5. At the division of the primary spermatocyte this element passes undivided to one pole thus producing two classes of secondary spermatocytes, one with nine and one with eight chromosomes.

6. The element in question is probably a bivalent chromosome formed by the union of two characteristic curved chromosomes which occur in spermatogonial and somatic cells. These elements may be seen to best advantage in the testicular or nephridial cells of 10 to 14 day chick embryos. The remaining chromosomes, typically 16 in number, are usually rod or block shaped.

7. In female chick embryos of 10 to 14 days, a relatively large percentage of dividing cells which were found in the ovarian and nephridial tissues showed a single large curved element.

8. Thus the evidence indicates that the male fowl is homozygous, the female heterozygous for this particular element.

9. The secondary spermatocytes when ready for division display, as a rule, four and five chromosomes respectively. The eight chromosomes which passed to the one secondary spermatocyte have paired to form four, and eight of the nine which passed to the other secondary spermatocyte have paired similarly, leaving the curved one unpaired.

10. The second division is regarded as not a reduction division since in the anaphase the daughter chromosomes often tend each to become bipartite or to resolve completely into two, thus revealing their dual nature.

11. Occasionally the pairing in the secondary spermatocytes is incomplete so that any number between four and nine may appear for division.

12. The odd element after lagging for some time at the equator of the spindle in the secondary spermatocyte, divides.

13. The spermatids which receive four chromosomes frequently pass on to one or more additional divisions. These are regarded as abnormal. Other evidences of degeneration in various spermatids indicate that a considerable number do not develop into normal spermatozoa. It seems probable that only one class of spermatids, that with the odd element, become spermatozoa.

14. The frequency distribution of head-lengths of spermatozoa in four different sets of measurements by two different observers shows no evidence of more than one class of spermatozoa.

15. The cytological evidence as presented in this paper harmonizes with the evidence derived from experimental breeding which shows the female to be heterozygous and the male homozygous for sex and sex-linked characters.

LITERATURE CITED.

Boring, Alice M., and Pearl, Raymond.

'14 The Odd Chromosome in the Spermatogenesis of the Domestic Chicken. Jour. Exp. Zool., Vol. 16, Jan.

Guyer, M. F.

'00 Spermatogenesis of Normal and of Hybrid Pigeons. Thesis, University of Chicago.

'09a The Spermatogenesis of the Domestic Guinea (Numida meleagris dom.). Anat. Anz., XXIV Band, No. 20 und 21.

'09b The Spermatogenesis of the Domestic Chicken (Gallus gallus dom.). Anat. Anz., XXIV. Band, No. 22–24.

'12 Modifications in the Testes of Hybrids from the Guinea and the Common Fowl. Jour. Morph., Vol. 23, March.

Jordan, H. E.

'11 The Spermatogenesis of the Opossum (Didelphys virginiana) with Special Reference to the Accessory Chromosome and the Chondriosomes. Archiv. f. Zellforsch., 7 Band, 1, Heft.

Smith, Geoffrey.

'12 Studies in the Experimental Analysis of Sex. Part 9—On Spermatogenesis and the Formation of Giant Spermatozoa in Hybrid Pigeons. Quart. Jour. Mic. Sci., Vol. 58, part 1.

Swift, Charles H.

'15 Origin of the Definitive Sex-cells in the Female Chick and their Relation to the Primordial Germ-cells. Am. Jour. Anat., Vol. 18, Nov.

Wodsedalek, J. E.

'13 Spermatogenesis of the Pig with Special Reference to the Accessory Chromosomes. BIOL. BULL., Vol. XXV., June.

EXPLANATION OF PLATES.

Plate I.

From photographs by the author. Enlargement 1,250 diameters unless otherwise specified. Photos 1 to 7 are from sections; the remainder, from smears.

Fig. 1. Polar view, metaphase of primordial germ cell in testis of thirteen-day chick; focused to show two characteristic curved chromosomes (one at the right and the other above and to the left); the other chromosomes, mostly out of focus, are rod-like.

Fig. 2. Tangential view, metaphase of primordial germ-cell in testis of thirteen-day chick focused to show two characteristic, curved chromosomes; the other chromosomes were out of focus.

Fig. 3. Showing the two curved chromosomes in a spermatogonium of an adult Rhode Island Red fowl.

Fig. 4. Showing a single curved element (to the right) in an early germ-cell in the ovary of a ten-day chick. The other chromosomes are out of focus but careful examination showed no other curved ones among them.

Fig. 5. Polar view, metaphase in nephridial tubule of a ten-day female chick. The single curved chromosome lies well to one side (above) the other chromosomes.

Fig. 6. Side view of an equatorial plate stage in an ovarian cell of a ten-day chick. A less deeply staining, curved element was attached to one edge (left) of the plate. The photograph does not reveal the curved condition which was readily visible under the microscope.

Fig. 7. Another ovarian cell in a ten-day chick; condition practically the same as in 6.

Fig. 8. Side view of a metaphase in a primary spermatocyte of a Plymouth Rock fowl showing the special curved element well toward one pole. × 1,300.

Fig. 9. Side view of a metaphase in a primary spermatocyte of a Langshan fowl, showing the curved element near one pole.

Fig. 10. Tangential view of a metaphase in a Rhode Island Red fowl showing the curved element near one pole of the spindle.

Fig. 11. Side view of a metaphase in primary spermatocyte of a Plymouth Rock fowl showing the curved chromosome.

Fig. 12. Ditto. The special chromosome is curved toward the observer. × 1,200.

Fig. 13. Ditto. The special chromosome is curved away from the observer and hence foreshortened in the photo.

Fig. 14. Side view, metaphase in primary spermatocyte of Langshan fowl, the curved element seen tangentially.

Fig. 15. Ditto in Plymouth Rock fowl.

Fig. 16. Showing nature of the spindle in primary spermatocytes (Plymouth Rock). The special element is not visible though probably in the equatorial plate in some such condition as in Photo 32 (polar view). × 1,200.

Fig. 17. Side view, metaphase in primary spermatocyte of Langshan fowl. The fuzzy appearance of the curved chromosome in this and various other photos is due to a coating of linin-like material which, though in sufficient contrast to the chromosome as seen under the microscope, photographs dark. × 1,300.

Fig. 18. Primary spermatocyte of Plymouth Rock fowl; the special chromosome curved away from the observer (see Fig. 24 for a different view of a somewhat similar stage).

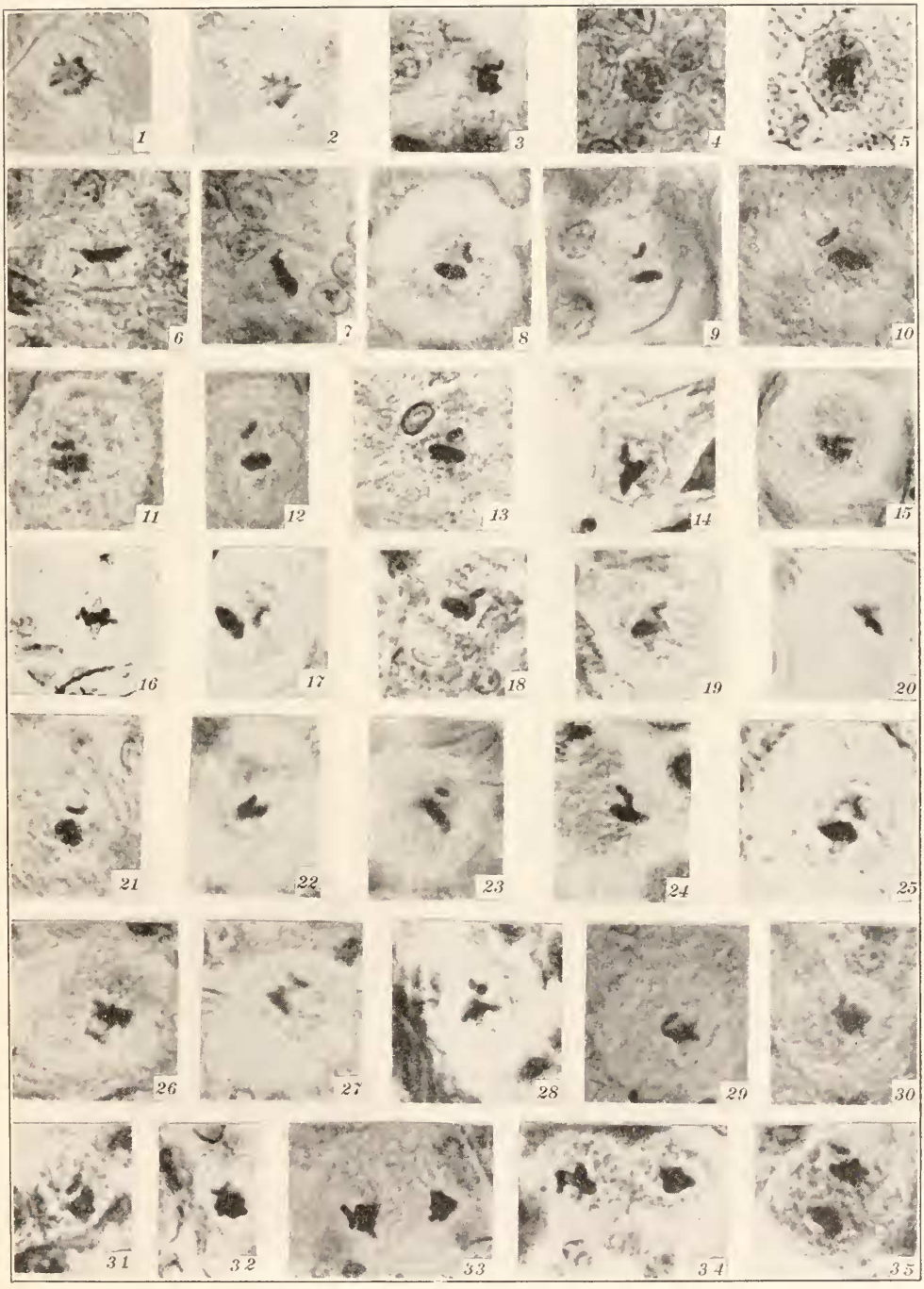

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FIG. 19. Primary spermatocyte of Langshan fowl. See comment under 17.

FIG. 20. Ditto. The curved element lies close against the other chromosomes in the equatorial plate stage and if the preparation were not greatly decolorized would be indistinguishable.

FIG. 21. The curved element in a spermatocyte of a Rhode Island Red fowl. × 1,200.

FIG. 22. Primary spermatocyte of Plymouth Rock fowl, side view of metaphase showing the curved chromosome lying just above the equatorial plate.

FIG. 23. Primary spermatocyte of Langshan fowl, the curved element seen tangentially and therefore foreshortened; easily seen to be the typical element when focus can be shifted.

FIG. 24. From Plymouth Rock fowl. See 18 for comment.

FIG. 25. From Langshan fowl. See 17 for comment. × 1,300.

FIG. 26. Ditto. × 1,300.

FIGS. 27, 28, 29. Showing various relations of the special chromosome to the spindle in primary spermatocytes.

FIG. 30. Polar view of metaphase in a Plymouth Rock fowl, the curved element lying at the upper edge.

FIGS. 31, 32. Ditto in Langshan and Rhode Island Red fowls respectively.

FIG. 33. Anaphase of a dividing primary spermatocyte in the Plymouth Rock fowl. The curved chromosome has passed over undivided to one pole.

FIGS. 34, 35. Stages somewhat similar to that shown in 33. In 34 the division has proceeded further; the cytoplasm is constricting to complete the division and the chromosomes are undergoing their second pairing, the group to the right having already formed a 4-group.

PLATE II.

From photographs by the author. Enlargement 1,250 diameters unless otherwise indicated.

All photographs in this plate were made from smears.

FIGS. 36–39. Metaphases in secondary spermatocytes of the Rhode Island Red fowl showing four chromosomes each.

FIG. 40. Side view of spindle in a secondary spermatocyte of the Plymouth Rock fowl; one of the four chromosomes has been displaced from its equatorial position, probably in making the smear.

FIG. 41. A four-chromosomed secondary spermatocyte of a Langshan fowl. × 1,200.

FIGS. 42–46. Side views of spindles bearing four chromosomes in secondary spermatocytes of the Plymouth Rock fowl.

FIGS. 47–52. Representative anaphases in the four-chromosomed type of secondary spermatocyte in Plymouth Rock (47, 49), Langshan (48, 50) and Rhode Island Red (51, 52) fowls.

FIGS. 53, 54. Equatorial plate stages in secondary spermatocytes of Langshan fowls showing four chromosomes each.

FIG. 55. Polar view of a metaphase in a secondary spermatocyte of a Plymouth Rock fowl showing five chromosomes.

FIG. 56. One end of an anaphase in a secondary spermatocyte which showed five chromosomes; two of them over lap in the photo so as to look like one.

FIG. 57. Side view of a five-chromosomed secondary spermatocyte in the Plymouth Rock fowl showing the fifth element, a curved chromosome, at one edge of the spindle. × 1,200.

FIGS. 58, 59. Stages in the Plymouth Rock similar to that shown in 57. In 58 the curved element lies at the left edge of the equatorial plate, in 59 at the right edge.

FIG. 60. Anaphase of a dividing five-chromosomed secondary spermatocyte of the Plymouth Rock fowl. × 900.

FIG. 61. Late anaphase of a dividing five-chromosomed secondary spermatocyte in a Plymouth Rock fowl. The five chromosomes at the lower pole while not distinctly visible in the photograph are just as distinct in reality as those at the upper pole. Fig. 169 is a camera lucida drawing of this cell which shows its true condition. × 1,450.

FIG. 62. Slightly different view of same preparation as shown in 61. × 950.

FIG. 63. A five-chromosomed secondary spermatocyte of the Plymouth Rock fowl, in process of division. The extra chromosome lags at the equator of the spindle until after the autosomes have divided, then it divides.

FIG. 64. A stage in the Langshan somewhat similar to 63. See Fig. 173 for a drawing of this cell.

FIG. 65. Side view of a *second* (anomalous) division of a four-chromosomed secondary spermatocyte in the Plymouth Rock fowl.

FIG. 66. Anaphase of such a cell as that pictured in 65.

FIG. 67. Side view of an equatorial plate stage in a four-chromosomed secondary spermatocyte of the guinea-fowl. × 1,150.

FIG. 68. Division figure (early anaphase) in an anomalous division of a spermatid in which two small-sized chromosomes are going to each pole. See Fig. 198.

FIG. 69. Material from Plymouth Rock fowl showing (above) an anaphase of a division of a four-chromosomed secondary spermatocyte, and also (below) an anaphase of a second (anomalous) division of such a four-chromosomed cell. × 1,300.

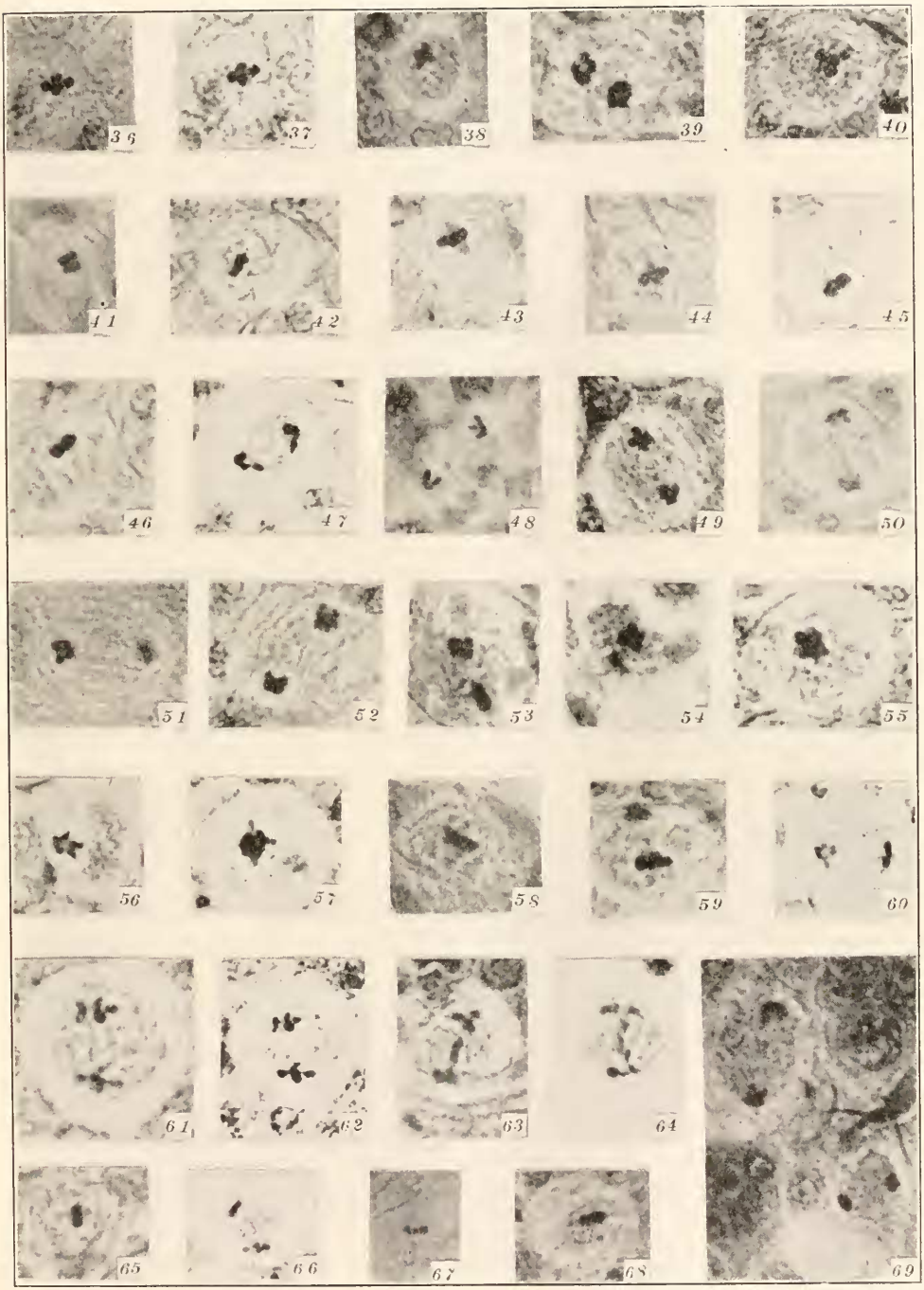
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Plate III

All drawings on this and the following plates are the work of Miss Hattie J. Wakeman. They were made with the aid of a camera lucida. Unless otherwise specified their magnification is approximately 2,000 diameters. All on this plate are from sections of male tissues. Figs. 72, 76, 81, 86, 87 and 95 are from the testes of chick embryos. All other figures except 79 are from cells in nephridial tubules of embryos.

Figs. 70–78. Polar views of division stages showing the two curved elements in various germinal (72, 76) and somatic cells of embryo chicks. In some a number of the autosomes have been cut away, in others all or most of the chromosomes are present. Fig. 76 is from a thirteen-day embryo, the others from ten-day embryos.

Fig. 79. Spermatogonium of adult fowl showing two curved chromosomes. The remaining chromosomal mass could not be resolved into individual elements.

Figs. 80–85. See remarks under Figs. 70–78. Fig. 81 is from a fifteen-day embryo, the others from ten-day embryos.

Fig. 86. Side view of metaphase in primitive spermatogonial cell of ten-day chick embryo. Two relatively huge curved elements are present at each edge of the equatorial plate.

Fig. 87. The same kind of cell and the same condition as in 86.

Figs. 88–96. Comment same as for 70–78. Figs. 88, 94, 96, from nephridial cells of ten-day embryos; Fig. 95 from testis of thirteen-day embryo.

Fig. 97. Anaphase in cell of nephridial tubule; each of the two curved elements has divided.

Fig. 98. A stage in a nephridial cell comparable to Fig. 86 of a primordial germ-cell.

Fig. 99. Probably a stage comparable to that shown in Fig. 97, only the curved elements from one side have been cut away.

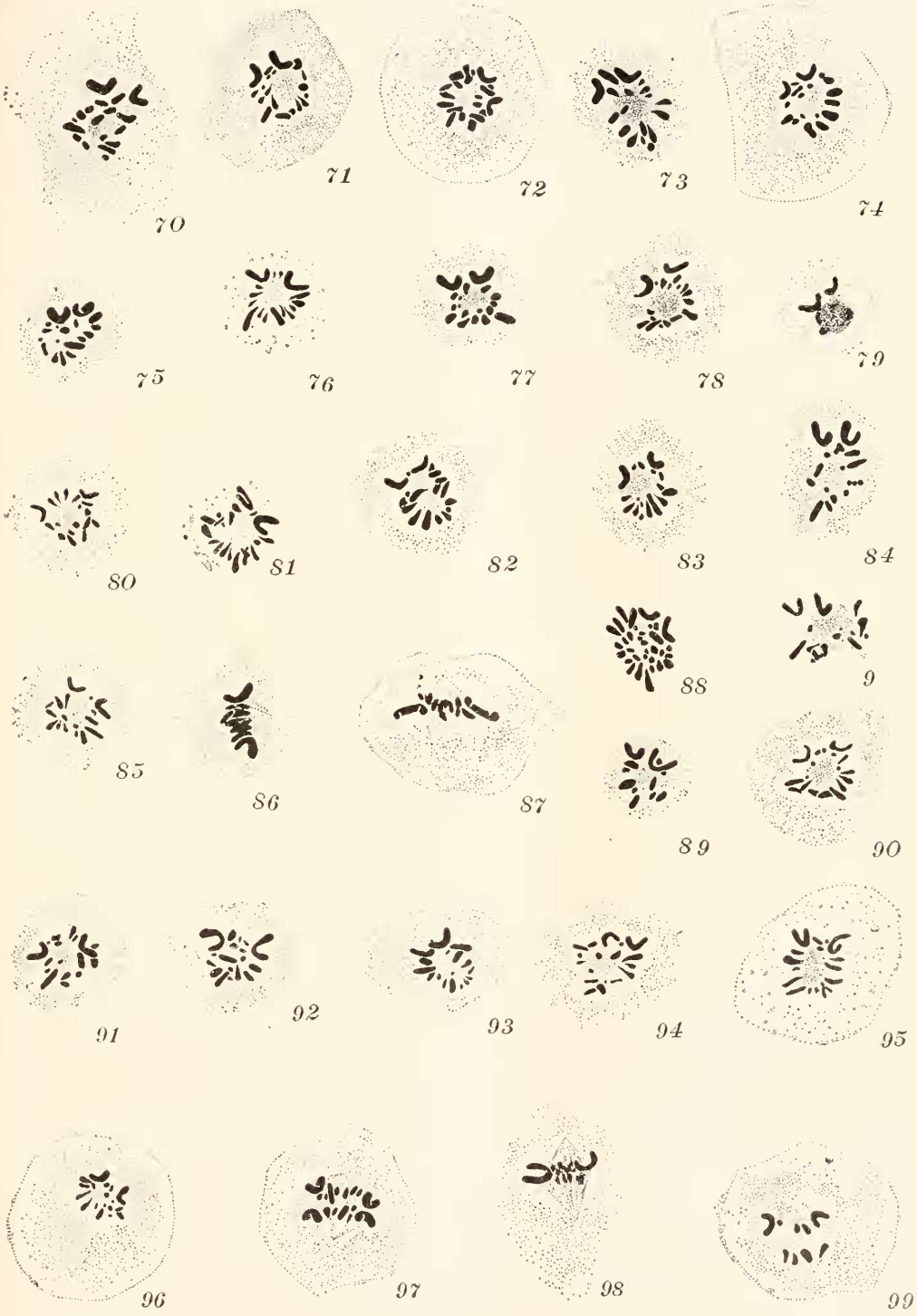
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Plate IV.

Figs. 100 to 117 are from embryonic ovarian or female somatic tissue, from sections; 118, 119 from sections of adult testes; 120 to 132, from smears. Magnification approximately 2,000 diameters.

Fig. 100. Polar view, metaphase in primordial ovum of ten-day chick, showing a single curved chromosome.

Fig. 101, 102. Polar view, metaphases from region of gonad in five-day chick.

Fig. 103. Showing curved element in nephridial tissue of ten-day female embryo.

Fig. 104. Metaphase of a dividing cell in the nephridial tubule of a ten-day female embryo; the single curved element lies at one edge of the equatorial plate; the long axis of the spindle lies across the short axis of the cell.

Figs. 105–108. Side views of metaphases in the germinal tissues of ten-day female embryos, each showing a single curved chromosome.

Fig. 109. Polar view, metaphase in nephridial tissue of ten-day female embryo.

Fig. 110. Division figure from germinal tissue of ten-day female embryo showing the curved element as a much lighter stained body than the autosomes.

Fig. 111. Early anaphase in a cell from the germinal tissue of ten-day female embryo showing the curved element just divided.

Fig. 112. A stage a little later than that shown in 111; from nephridial tubule of same embryo.

Fig. 113. Curved element in metaphase, nephridial tubule of thirteen-day chick.

Figs. 114, 115, 116. Side view of division figures from ovarian tissue of thirteen-day embryo, each showing a single, long, special chromosome. In 115 the chromosome was much lighter in color than the other chromosomes.

Fig. 117. Side view of division figure in a cell from the nephridial tubule of a thirteen-day female embryo; the curved element has divided.

Fig. 118. Nuclei of spermatogonia as seen in thin sections from the testis of an adult fowl. Two elongate nucleolar-like bodies are to be seen in each. The urved nature of these bodies suggests that they may be the same as the two curved elements which appear at division time.

Fig. 119. Nucleus of late spermatogonium or early spermatocyte showing two nucleoli and general granular appearance.

Figs. 120, 121. Nuclear phases of the early growth period of primary spermatocytes.

Fig. 122. Nucleus showing synizesis in a primary spermatocyte.

Fig. 123. Post-synizetic stage; two elongated, nucleolar-like bodies are present.

Fig. 124. Nucleus showing heavy spireme which immediately precedes the formation of chromosomes in primary spermatocytes.

Figs. 125–132. Nuclei showing transition stages between the breaking up of the spireme and the formation of the chromosomes in primary spermatocytes. The difference in size of the nuclei is probably due to different degrees of flattening in making the smear rather than to an actual difference. Fig. 132 shows various tetrad-like groups.


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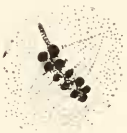
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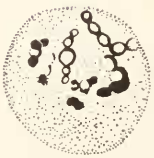
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PLATE V.

Figures 137, 138, 141, 145, 148–150 are from the Langshan, Fig. 139 from the Rhode Island Red, and the others from the Plymouth Rock fowl. Magnification approximately 2,000 diameters. With the exception of 139 all the drawings are from smears.

FIGS. 134–149. Side views of division stages in primary spermatocytes showing characteristic positions and appearances of the large curved chromosome which, like a typical X-element, passes undivided to one pole of the spindle and thus gives rise to two classes of secondary spermatocytes, one with nine and one with eight chromosomes.

FIGS. 150, 151. Anaphases of divisions in primary spermatocytes showing the curved chromosome associated with but one set of the daughter autosomes.

FIG. 152. Polar view of metaphase in a primary spermatocyte showing the special curved element at one side of the chromosome mass.

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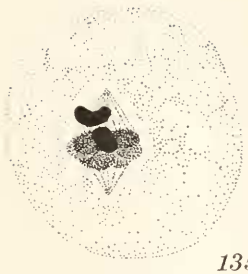

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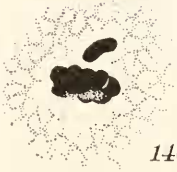

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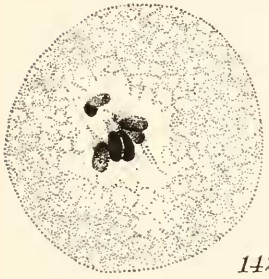

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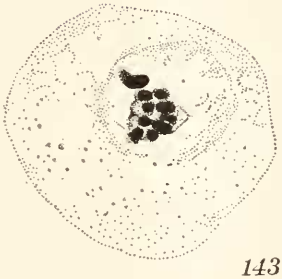

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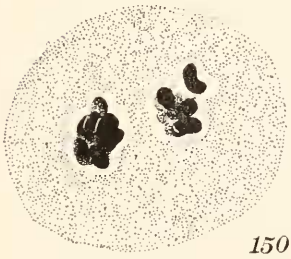

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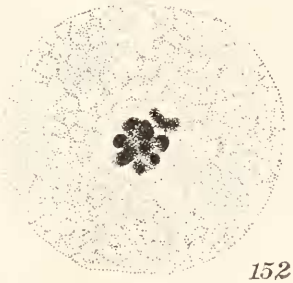

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Plate VI.

All drawings are from smear. Figs. 168, 169, 171 are from Plymouth Rock, Figs. 153, 163–166, 170 and 174 from Langshan, and the remaining figures except 160 are from Rhode Island Red fowls. Magnification approximately 2,000 diameters.

Figs. 153–158. Polar views of equatorial plate stages showing various relations of the special curved chromosome to the other chromosomes.

Fig. 159. Side view showing same.

Fig. 160. Polar view of an equatorial plate stage in a primary spermatocyte of the guinea-fowl. In the guinea the accessory chromosome is characteristically comma-shaped.

Fig. 161. Equatorial plate stage in a secondary spermatocyte, showing five chromosomes.

Fig. 162. A five-chromosome and a four-chromosome group lying in a common mass of cytoplasm each ready for division as a separate nucleus.

Fig. 163. Side view of an equatorial plate stage in a secondary spermatocyte, showing five chromosomes, one of which is the curved chromosome received from the primary spermatocyte.

Fig. 164. Telophase of a dividing primary spermatocyte showing secondary pairing of the daughter chromosomes in preparation for the next division. The eight ordinary chromosomes at each end pair to form four; the extra curved chromosome remains unpaired.

Figs. 165, 166. Side views of metaphases in secondary spermatocytes showing curved chromosomes at one edge.

Figs. 167–174. Various anaphase stages in the division of the five-chromosomed secondary spermatocytes. In 168 and 172 the double nature of the autosomes is indicated by their bipartite appearance. The lagging in division of the fifth chromosome is indicated in 167, 168, 172–174.

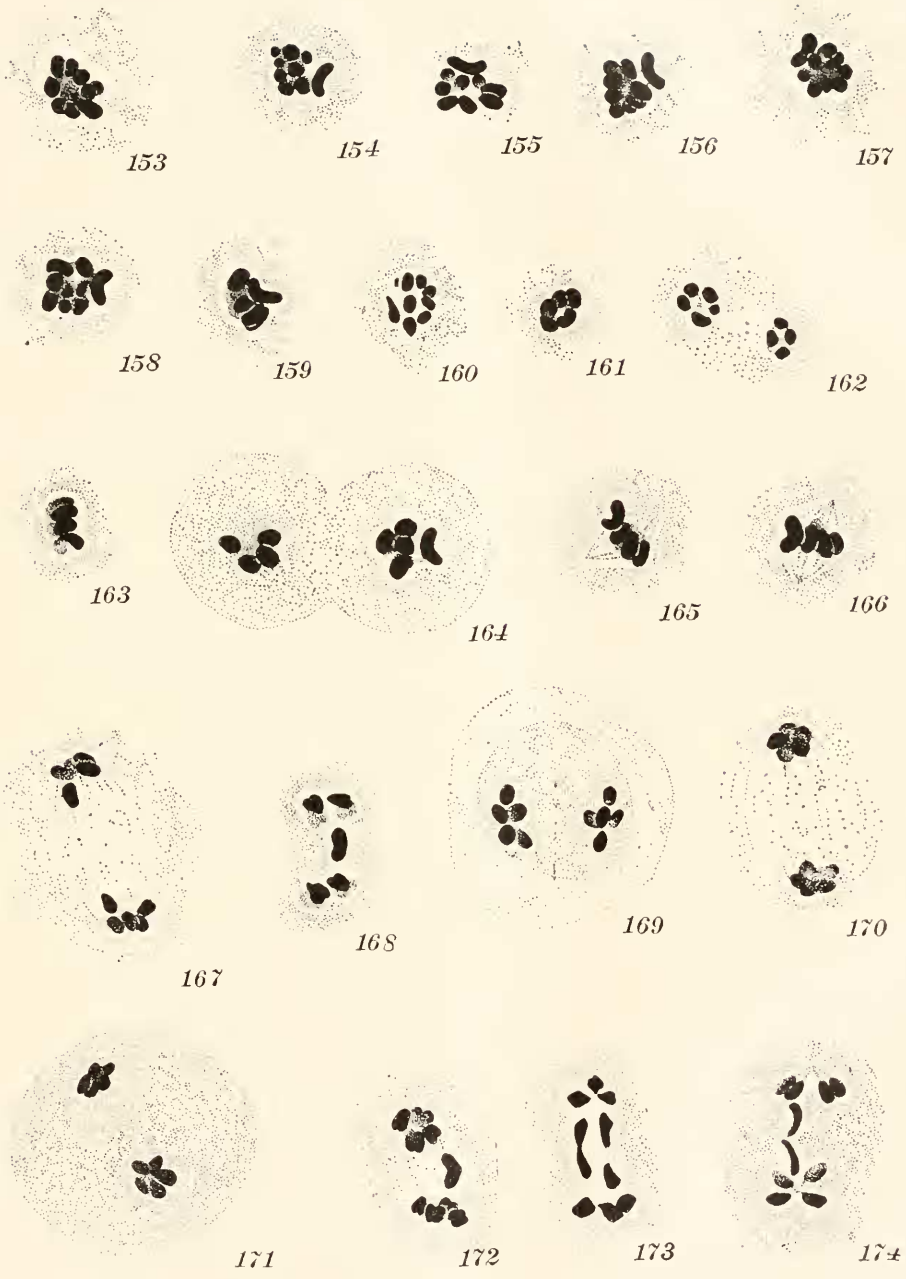
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Plate VII.

All drawings except 181, 199 and 200 were made from smears. Figs. 176, 180, 182, 183, and 186 are from the Langshan, Figs. 175, 177–179, 181, 184, 185, 187 and 188 are from the Rhode Island Red, Figs. 189, 190 from the Guinea and the remaining figures from the Plymouth Rock Fowl.

Figs. 175–183. Characteristic metaphases of secondary spermatocytes with four chromosomes. The double nature of the individual chromosomes is shown in 180.

Figs. 184, 185. Secondary spermatocytes showing five chromosomes. Two of the chromosomes are small and are probably comparable to one of the larger ones, having failed to unite.

Figs. 186–188. Anaphases in secondary spermatocytes showing four chromosomes at each pole.

Figs. 189–191. Secondary spermatocytes from the guinea fowl showing four chromosomes.

Fig. 192. Probably a second (anomalous) division in a secondary spermatocyte. Both spindle and chromosomes are reduced in size.

Fig. 193. A first and a second division of four-chromosomed secondary spermatocytes which lay side by side in a smear. The first is regarded as normal, the second is probably anomalous.

Figs. 194–197. Various phases in the division of the smaller sized (probably anomalous) four-chromosomed cells.

Fig. 198. Anomalous division figure showing still further reduction in number and size of chromosomes than that shown in 197.

Fig. 199. Nucleus of resting spermatid.

Fig. 200. Typical appearance of normally transforming spermatids.

Fig. 201. Conditions comparable to those shown in Fig. 193.

Fig. 202. A stage similar to that shown in 195.

Fig. 203. Normally transforming spermatids.

Fig. 204. Spermatids which are probably abnormal or degenerating.


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SPERMATOGENESIS OF THE DRAGON-FLY SYMPETRUM SEMICINCTUM (SAY) WITH REMARKS UPON LIBELLULA BASALIS

ELIZABETH A. SMITH,

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CONTENTS.

Observations upon the changes which occur during the ripening of the male sex products in *Sympetrum semicinctum* (Say) are described in this paper. The account, beginning with the last multiplication division of the spermatogonia, traces especially the behavior of the chromatin through the growth and maturation periods up to and including the formation of the spermatozoa.

The sex-chromosome, a round, univalent body, appears at the beginning of the growth period and by its different staining capacity can be traced as a definite body throughout the changes

which take place in the nucleus, from the pre-synaptic period up to the formation of the spermatid, where it loses its identity and combines with the chromatin derived from the autosomes. The exact behavior of the autosomes is difficult to make out in all details. They apparently unite by a side to side union, or parasynapsis, and later separate along the line of union to form rings which condense into quadripartite elements.

For comparison, another species of dragon-fly, *Libellula basalis*, was also studied.

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I. Nymphs.

Several hundred nymphs of two species, *Sympetrum semicinctum* and *Libellula basalis*, were collected in the spring and fall of 1912. Most of those obtained in the spring were found in the ooze of the marsh before they had come up for transformation. In the fall, the majority were found clinging to reeds and roots of grasses along the bank of a little stream on the University farm. The most prevalent form was *Sympetrum semicinctum* (Say). See Fig. 1, for a drawing of a nymph. A large number of nymphs secured early in the spring, 8 to 10 mm. long, must have belonged to the brood of the preceding year. Other nymphs taken in the spring measured 17 or 18 mm. Some of these had well-developed testes and were about ready to transform, while others had no visible gonads. Of the nymphs obtained in the fall, many were small and had evidently hatched from eggs laid during July or August, while most of the large ones had reproductive organs just developing. These facts indicate that the larval period extends over more than one year.

II. Testes.

The testes of the nymphs appear as two white filaments, one on either side of the digestive tract, extending almost the entire length of the abdomen. They are not composed of lobules like

the testes of certain other insects, but consist of globular cysts arranged one or two layers deep around a central duct which runs through the organ in more or less of a zig-zag course. Each testis is of nearly uniform size along its entire length, tapering at the posterior end where the vas deferens emerges. The epithelium covering it is thin and hidden by a layer of fatty tissue which contains tracheæ. The central duct has a thick epithelial wall and each cyst, surrounded by a thin layer of epithelium, apparently opens into the central lumen by a separate duct. All the developing stages of the spermatozoa may in favorable preparations be found in one cross-section. The cysts seem to have no definite arrangement in the tubule according to the age of the products. One containing primary spermatocytes may lie between two containing mature spermatozoa. As a rule all the cells in each cyst are at the same stage of development, although two or three primary spermatocyte divisions may occur occasionally in cysts containing older products. Where the cell is passing rapidly through the later prophase stages which precede the first maturation division, cysts containing two closely successive stages occur. In the older cysts which contain spermatozoa, there is a shrinkage in size and the cysts become separated by large spaces. Fig. 2, a cross-section of a whole testis, shows the central duct with its thick epithelial wall; the cysts containing products at different stages of development; and the fatty layer containing the tracheae and covering the outer epithelium of the testes. In some testes degeneration had taken place in a few cysts containing spermatogonia. Fig. 3, a cyst in which degeneration had taken place, shows how the chromatin condenses into a solid mass in the nucleus of each spermatogonium.

In the female larva, the ovaries lie in the anterior end of the abdomen, dorsal to the digestive tract and are close together anteriorly, while posteriorly they separate forming an inverted V.

III. Method of Fixing and Staining.

Injection of the living larva with the killing fluid was found to be the best method of killing and fixing the gonads. A hypo-

dermic syringe with a small needle was used. The dorsal part of the abdomen was then cut off and the nymph placed in a dissecting pan of water. The testes were removed as quickly as possible to a vial containing the fixing fluid used for injection. Where Bouin's fluid was used for killing, the dissection was made in 70 per cent. alcohol instead of water. The best results for working out the different stages, especially those of the growth period, were derived from fixation in Bouin's fluid followed by Heidenheim's iron-hæmatoxylin with eosin or acid fuchsin as counter stains. Some excellent preparations were obtained by staining with an aqueous solution of safranin for two minutes, followed by lichtgrün. Good results also followed the use of a saturated aqueous solution of Gentian violet and orange G after fixation with Flemming's solution.

For quick observations upon fresh material aceto-carmine was used. Cells stained with this swell slightly but cytological details such as chromosomes, spindle, centrosome and spireme are brought out clearly. Satisfactory counts of polar views and camera-lucida drawings could easily be made. The details obtained in these entire cells could be used as a check in examining sections.

Testes were also teased and mounted unstained upon a slide in Ringer's and in physiological salt solutions. Normal saline caused plasmolysis after a short interval, but tissue placed in Ringer's solution made up with a .5 per cent. normal salt instead of .7 per cent. remained normal for several hours before signs of disintegration began. The chromosomes in both *Sympetrum* and *Libellula* could be seen in the fresh material, as they have a refractive power which differs from that of the cytoplasm. The chromosomes upon the spindles both in the metaphase and anaphase could be distinguished as separate bodies in the primary spermatocyte division. In growth stages a spireme was visible while in the spermatogonia the chromatin nucleolus was evident. This proves that the details of the cell, as revealed in preserved materials, are reasonably faithful presentations of the conditions which really prevail in the living cell. Complete cell division was not observed. Cells teased apart remain connected by long protoplasmic threads. The fact that the chromosomes

can be separately distinguished opens up the possibility of experimentation upon cell division when a solution isotonic with the body fluid of the dragon-fly nymphs can be determined.

IV. Spermatogenesis of Sympetrum semicinctum (Say).

(*a*) *The Spermatogonial Period.*

The length of larval life in this form is unknown and it probably varies with temperature and food supply. Nymphs obtained early in May this year which measured 1 cm. in length were at least one year old for the adults had not begun to emerge. These nymphs possessed no visible gonads. In the youngest larvae bearing reproductive organs, it was exceedingly difficult to find spermatogonial divisions. Until the life cycle is known no explanation can be offered for this, but it may be that the gonads develop during the winter of the second year of larval life after the insects are in the mud at the bottom of the streams where it is hard to find them. The material of this particular species was difficult to work with on account of: (1) the small size of the cells and their closely crowded condition; (2) the considerable number of chromosomes; (3) the irregular arrangement of the cysts as to age, referred to under III.

The spermatogonial cells could be easily distinguished by their large nucleoli and their large-mesh nuclear network. The chromatin granules, sparsely scattered along the linin network in the center of the cell, were collected in small clumps close to the nuclear membrane. This arrangement of the chromatin gave to the nucleus a clear appearance. Frequently small chromatin bodies appeared in the network, but these were inconstant in number and apparently of no importance in the later development. The nucleolus appeared more often as composed of a clear ground substance, probably linin, in which masses of chromatin were imbedded. It sometimes looked like a solid chromatin mass. Lewis and Lewis ('15) found that in a living cell, the nucleolus was never a compact body, but was coarsely granular and large in proportion to the nucleus. The degree of contraction of the ground substance inclosing the granules, depending upon the fixation of the material, would account for

the difference in appearance. The cytoplasm in some cells was clear and homogeneous, while in others it was alveolar and in most cases formed only a narrow sheath around the nucleus. Fig. 4 shows groups of spermatogonia with the structures just described, nucleolus, chromatin body, nuclear network and alveolar cytoplasm. Fig. 5 represents a spermatogonial cell from a smear preparation in which the chromatin granules appear as clumps near the nuclear membrane. Fig. 6 shows the 25 spermatogonial chromosomes in an aceto-carmine smear.

In the spermatogonial divisions, the undivided chromosomes could rarely be distinguished, as all the chromosomes in the metaphase usually blend into a black compact mass. In Fig. 7, a polar view of the metaphase before the last spermatogonial division, a few of the separate chromosomes can be seen. They are dumbbell-shaped, varying in size and are not as large as those found by McGill ('04), in *Anax junius* in the same stage. In some of my own preparations of *Anax junius* there were clear polar views in which 27 chromosomes could be counted. This verifies the corrected count of Lefevre and McGill ('08). In *Sympetrum semicinctum*, however, it was impossible to find many cells in which the chromosomes were sufficiently separated to permit of a satisfactory count. In many instances where partial counts were possible, more than twenty could be distinguished. Fig. 8 shows twenty-five plainly in a polar view of a telophase, and while the two cells in which twenty-five could be distinctly counted do not afford sufficient evidence from which to draw a conclusion, judging from the reduced number found in the primary and secondary divisions, the correct spermatogonial number should be twenty-five. The dense appearance of the polar view is due to the deeply staining proclivity of the threads which connect the chromosomes.

Figs. 9, 10, and 11 are telophase stages of the last spermatogonial division, showing approximately the amount of chromatin going to each cell. The telophase stages are more abundant than the metaphase ones in my material. A polar view of the telophase shows 25 chromosomes distinctly in one case and many show plainly 21 chromosomes and there are indications of others

which are not in focus. Figs. 12 and 13 show polar views of telophases of the last spermatogonial division.

The chromosomes soon fray out into indistinct masses as in Fig. 14. One, however, remains distinct and round in greatly destained preparations and may appear divided in some instances. Figs. 15 and 16 show the round chromosome as a single and as a double body. It is easy to distinguish this stage from the spermatogonium as the chromatin is thickly scattered throughout the cell in indistinct, granular masses. Some of these masses aggregate in the center or toward one side of the nucleus to form a nucleolus and the round body then is indistinguishable, obscured probably by the masses which formed the nucleolus (Fig. 17). There is then formed a vague, indefinite reticulum: the inconstant chromatic bodies mentioned as sometimes present in the spermatogonia are never present here. Wilson ('12), in stage A. in *Oncopeltus*, which is similar, says that in the early telophase, the sex chromosomes cannot be identified while in a little later stage, the sex chromosomes are elongated and the autosomes form a lightly staining, vague net-like structure in which individual chromosomes cannot be distinguished. Davis ('08) and McClung ('02*b*) also describe a like stage in the Orthoptera.

(*b*) *The Changes Occurring in the Growth Period up to and Including Formation of Crosses.*

As to exactly what takes place in synapsis it is hard to assert positively. There is present throughout this period a round, compact, deeply staining chromatin body which can be traced in every stage. This never loses its identity, though it may be obscured in some cells and from its subsequent behavior it can be identified as the sex-chromosome. It seems reasonable to suppose that it may be identical with the dense round body present at the end of the last multiplication period. But the disappearance of this body in the rest stage, although possibly only hidden under chromatin masses, breaks the continuity between the end of the last spermatogonial period and the growth period.

1. The nucleus has increased slightly in size and there is a corresponding increase in chromatin material, which is arranged into large, deeply staining, irregular masses. That the cell remains with the chromatin in the diffuse condition into which it passed at the end of the spermatogonial division for only a short time seems evident from the fact that few cells are found in that stage. Correspondingly large numbers contain the deeply staining masses. These masses are entirely different in appearance from those formed at the end of the multiplication period by the fraying out of the univalent, spermatogonial autosomes; they are larger, more irregular and stain more deeply. Their formation is not clear, the nucleolus separates into the chromatin particles which were loosely incorporated into it; the granules in the faintly staining network aggregate into clumps and the chromatin while increasing in bulk, develops a greater capacity for staining. Part of the process is a reversal of the behavior at the end of the spermatogonial division. These masses are so closely crowded that the number cannot be counted with certainty, but it is about equal to the diploid number. Most of them have ragged, irregular edges (Figs. 18, 19 and 20). One, marked X, stands out distinctly on account of its round, smooth outline and this later becomes the sex-chromosome. Toward the close of the stage, slender threads begin to extend out between the masses.

2. *Leptotene Stage.*—Each mass formed in the preceding stage ultimately becomes converted into a slender thread through the gradual outward migration of its component granules (Figs. 21, 22). The conditions correspond to that found by Montgomery ('11) in *Euchistus*, and by the Schriners ('06, *a* and *b*) in *Tomopteris*, *Spinax* and *Myxine*. The whole nucleus is now filled with fine, granular, much interlaced threads and it is impossible to determine their number. It may be that each mass only forms one thread, in which case there would be 24 threads which corresponds with the diploid number minus one.

The sex chromosome (Fig. 23) can be distinguished by its more compact make-up and more regular outline. It retains its shape and staining capacity, after the nucleus is filled with threads.

3. *Synapsis.*—In general, the leptotene threads are so scattered and tangled throughout the cell that it is impossible to be sure of their exact behavior. In occasional cells of a section where only a few threads remain, some threads can be seen to lie parallel to each other, while other threads are united at one end into V's (Fig. 24).

4. *Synizesis.*—All the threads drift to one side of the nucleus and what appears to be a continuous spireme is formed. If the leptotene threads paired previously side by side as they seem to do in synapsis, the spireme is formed by the end to end union of such paired threads. At this stage, the spireme thread is double (Fig. 27) and the two component threads are twisted and interlaced (Figs. 25 and 26). The spireme then becomes denser and thicker, possibly by the contraction of the loops which are closely drawn together near the side of the nucleus. The typical bouquet stage described by many investigators appears. There are in polar views through the loops 24 cut ends and as each loop is doubled back so that it would be cut through twice, the actual number of loops is twelve, which corresponds to the haploid number of autosomes.

The sex-chromosome is still a round, compact body and is seen best when it lies out near one of the larger loops. As a rule it is obscured in the loops near the nuclear wall.

The centrosome first appears at this stage and in some cells is divided. In others the two centrosomes are separated though there is no indication of the spindle. A large plasmosome sometimes appears in the cytoplasm as early as stage 1, but it is more common in this period. Figs. 28 and 29 show the spireme and Figs. 30 and 31 are polar views to show the cut ends. In Fig. 29 the sex-chromosome is shown among the loops of the spireme.

5. *Segmentation of the Spireme.*—The thickened spireme spreads out and breaks up into parts or segments, which are curved into horse-shoe shape loops. The segments are less than the diploid number, but it is not possible to count them accurately. A segment sometimes shows a split that is presumably the space between two leptotene threads which entered into its composition. That these are the same two threads which paired originally seems indicated in Fig. 32, where the

threads are twisted around each other into a chiasma. Jansen ('09) found that the threads of a tetrad became twisted and his figures resemble closely Figs. 32 and 33. The sex-chromosome is unaltered.

6. *The Condensation of the Segments into Crosses.*—The curved segments of stage five, now open up along the plane of the split to form loops and by becoming disunited at one end V's and U's occasionally occur. If the segments are extremely curved when the two threads split apart, rings bent in the form of eights are produced (Fig. 34). These do not correspond to the eights which some workers derive from such gemini because the threads are everywhere separated except at the two ends. An eight corresponding to one formed from a geminus sometimes results when the two component threads of a segment while remaining attached in the middle and at the ends, separate between the middle and the ends. Fig. 35 shows a true eight in the nucleus of a cell and the resemblance to the bent rings in Fig. 34 is apparent. The threads of each segment are granular and appear much like the leptotene threads of stage 2. In my preparations of *Anax junius* the chromomeres are plainly distinguishable in the threads (Fig. 36).

This stage is much like the prophase of the Orthopterans described by McClung ('14). Among the opened out loops, there are many modifications. The most striking one is the signet ring. If the two threads of a segment divide along the synaptic plane (a point which will be discussed more in detail later) a ring is formed which is composed of two autosomes united at both ends and pulled apart in the center. Now if a secondary split takes place in each thread and the two halves become separated at one of the synaptic ends, the signet ring type is explained. When viewed from the side such a ring appears as a loop with crossed ends (Figs. 37 and 38). By far the larger number of segments open out into true rings without this modification and this type was not found in every nucleus.

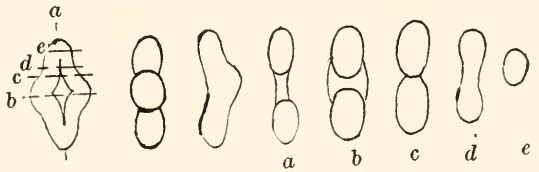
The two autosomes after opening out to form a ring remain connected at the ends. The granular threads condense into more compact ones and each apparently bends out in the middle in opposite directions until the connected ends which were far

apart are brought close together. At first the long axis of the loop coincided with the plane of union in synapsis, but with the bending of each autosome the long axis is reversed and lies at right angles to the original long axis. The ends where the chromosomes united do not bend, but remain extended to form side arms. A cross is thus produced by this process, the upper half composed of one univalent autosome and the lower half of another. When the signet ring condenses it assumes the same form as the others; all finally become condensed crosses showing a less dense area in the middle.

Figs. 39, 40, and 41 show cells containing a number of crosses, and in Figs. 40 and 41, the sex-chromosome which has retained its round compact form through all the stages of the growth period appears. In some prophases as many as nine different sizes of crosses which would form bivalent chromosomes of the primary spermatocyte could be counted. The largest bivalent autosome comes from a cross in which the secondary long axis is much extended and the united ends of the univalent autosomes bulge only slightly to form exceedingly short side arms (Fig. 41).

(*c*) *First Spermatocyte Division.*

The chromosomes, as last described, have become bivalent masses; with only an area less dense in the center to indicate the former central split. As just described, each bivalent has four projecting parts corresponding to the arms of a cross. Hence, if a bivalent is cut exactly in two, one half when viewed



TEXT-FIG. 1.

from the cut surface shows two ends connected by a cross piece below the level of the ends. It is difficult to give an adequate idea by a description but the text figure makes this clear by picturing the result in cross-section of different cuts through the bivalent.

Usually, in a polar view, the chromosomes appear bipartite or round because the cut does not exactly halve the bivalents. The sex-chromosome in such a stage of nuclear change is round and shows no evidence of a constriction. Figs. 42, 43 and 44 are polar views of the twelve bivalents and the single univalent sex-chromosome.

In *Sympetrum* there is usually an outer ring of seven bivalents and the sex-chromosome, surrounding five in the center. The chromosomes vary in size somewhat, although five are of nearly uniform size; one is larger and the others grade down gradually to the smallest which is smaller than the sex-chromosome. In some cases the latter may appear to one side, and then only four bivalents occur in the middle, one of the central bivalents apparently replacing the sex-chromosome in the outer ring. In a side view of this stage, all the autosomes are compact bivalents with four shortened arms and a central clearer area. The central bivalents can be seen below the others in an exact metaphase. The sex-chromosome can be easily distinguished from the bivalents by its rounded appearance and clear vesicle-like zone around it. All are connected with double spindle threads. The centrosomes are extremely large. The bivalents when dividing pull into two halves, presumably along the line of junction of the two ends of the autosomes which agrees with McClung's statement that separation between the parts of bivalent chromosomes is more likely to occur along spaces between whole chromosomes.

The bivalents do not seem to behave like tetrads when dividing. Occasionally they pull out into the form depicted in Fig. 48, but when the telophase is examined the autosomes show no split. If true tetrads were formed the telophase number of autosomes would be doubled or each autosome would be bipartite. Fig. 49 shows the sex-chromosome lagging behind the others and in Fig. 50 it is dividing after the others are well on their way to the poles. Figs. 51 and 52 are telophases of this division. Usually in later stages of division the sex-chromosome cannot be distinguished from the autosomes, but it occasionally stands out in a polar view of a telophase as in Fig. 53. In the telophase, the chromatin is massed at each pole, the cytoplasm

becomes constricted and the cells are divided. As in Fig. 51 a few spindle fibers may connect the two cells.

This is probably the true reduction division for the bivalents. For the sex-chromosomes which splits equally into two parts, this is an equational division.

(*d*) *The Second Spermatocyte Division.*

In the telophase of the primary division, the univalent autosomes are closely crowded together, but they soon separate and become scattered around the edge of the nucleus in preparation for the second division which closely follows the first. By actual measurement these cells are one half the size of the cells in the prophase of the preceding division. The univalent autosomes are small and compact. The second division occurs without any intermediate stages, the same autosomes which were in the preceding telophase become the metaphase autosomes. In polar view, they are round with no evidence of constriction and are arranged in the following order: a ring of 9, enclosing three, of which one is very small. This is nearly the same order as that occurring in the metaphase of the first division. The sex-chromosome, however, always lies outside the ring and is surrounded by its usual vesicle. This order prevailed in all cases counted although the size of the ring varied; the autosomes were spread apart more in some cells and in others they were collected into a smaller ring. In fact polar views of this stage (Figs. 54 and 55) appear much like the telophase of the first division. In Figs. 56 and 57 the sex-chromosome is not visible.

In side views of this stage, all the autosomes are dumbbell-shaped, with the two halves longer than broad. The sex-chromosome is at one side of the spindle and can be identified by its roundness, its vesicle and the lack of spindle fibers. During division, the sex-chromosome precedes the dividing autosomes and, undivided, goes to one pole. It may reach the pole before the autosomes start their division or it may be only a little in advance. In no spindle observed did it ever lag behind the others. Figs. 58, 59, 60, 61, 62 and 63 show spindles of the second division, with the sex-chromosome in various positions. In late telophases some of the spindle fibers may clump together

at the point where the transverse constriction of the cytoplasm takes place and a thickened thread results. This is shown in Fig. 64 which is a telophase. Fig. 65 is a drawing from an aceto-carmine smear in which the sex-chromosome stood out plainly at one pole. The centrosomes when present are much smaller than in the preceding division and in many cells they cannot be seen.

If the first division was reductional as the evidence seems to show, then theoretically this division must be equational. The sex-chromosome passed over undivided and this represents the reduction stage for it, as it divided equally in the first division. In *Libellula basalis* the sex-chromosome goes over undivided in the first division. Sutton (1900) found also that in *Brachystola* the sex-chromosome passed over undivided in the first division, so that as regards this element in different species, it is obvious that the place of reduction is not always the same.

(*e*) *Transformation of the Spermatid.*

At the end of the second maturation division each of the spermatids resulting from one secondary spermatocyte contains a mass of chromatin which never resolves itself into individual chromosomes. In many cases after the two daughter spermatids from one secondary spermatocyte are completely separated, the sex-chromosome stands out distinctly from the chromatin mass in one of the cells as indicated in Figs. 66 and 67. In most spermatids, the sex-chromosome is incorporated into the chromatin mass and there is no noticeable difference in the amount of chromatin in the two classes of spermatids. The chromatin becomes broken up into a number of irregular masses, three or more, connected by a faint network containing chromatin nodules at the intersections of the meshes (Figs. 68, 69, 70, and 71).

At this stage, the centrosome which lies close to the nuclear wall sends out a fine thread which is the axial filament of the tail. The cytoplasm at the end opposite the centrosomes elongates into a head spine which is free from granules and attains a considerable size (Figs. 72 and 73). Bütschli as far back as '71 described in the spermatozoa of *Agrion puellæ* a

head-spine which after increasing from .01 mm. to .45 mm. in length diminished with the ripening of the spermatozoa until it was only .0078–.009 mm. long. Fig. 74 is a drawing from a smear preparation showing the shape of the nucleus and the elongated head spine in the spermatid. The nucleus which at first is round, elongates and apparently enters the clear zone comprising the head-spine. The cytoplasm becomes a thin sheath around the nucleus which is extremely long and narrow. There is never a distinct middle piece, but the knob around the centrosome may be homologous to the middle piece found in other spermatozoa. In the ripe spermatozoa, this does not appear separated from the nucleus and the whole head stains as if composed of solid chromatin. The chromatin, however, is collected around the outer side of the nucleus, as cross-sections (Figs. 75 and 76) show a denser staining band of chromatin just beneath the nuclear membrane. The adult spermatozoa are motile and swim along with a twisting spiral motion. Figs. 77, 78, and 79 are drawings from aceto-carmine preparations in which the spiral twist of the adult spermatozoön is indicated.

There are apparently no external differences among the spermatozoa. In many spermatids drawn to scale no variation in size of head could be detected. Zeleny and Faust ('15) have found a dimorphism in the spermatozoa of *Æschna canadensis*, the ratio of the two 1.00 : 1.03. It is possible that using their method a dimorphic curve could be plotted for *Sympetrum*. However, the sperm on account of their method of locomotion would be fixed in varying twisted postures. When these are stained, their twisted condition is noticeable in only a few cases; most of the stained specimens appear as rods. This fact alone might cause such a discrepancy in size among the adult spermatozoa as to be mistaken for dimorphism even should the latter not occur. In *Sympetrum* from the mode of formation, one half the spermatozoa has more chromatin than the other half and this extra chromatin brought in by the sex-chromosome probably causes a physiological difference even if there is no visible dimorphism.

V. Some Notes on Libellula basalis.

The chromosomes of *Libellula basalis* in a general way undergo the same changes described for *Sympetrum*. The cells are larger; the diploid number of chromosomes is twenty-five, the reduced number thirteen. From the fact that certain cysts contain as few as eight large spermatogonia, while others contain large numbers of smaller ones, it is evident that several spermatogonial divisions occur. In several cysts in which an attempt was made to count the later spermatogonia, from 45 to 100 were present. The nucleus of a spermatogonium is usually eccentrically placed in the cell and part of the chromatin forms a large irregular nucleolus which is connected with chromatin nodules around the periphery of the nucleus by a few faintly staining threads. Fig. 80 shows such a cell from an aceto-carmine preparation, while Fig. 81 represents another from a section stained in iron-alum hematoxylin; Fig. 82 is a spermatogonium drawn under low power and is of interest since it was taken from a living cell in salt solution. In all the cells examined in this way, the protoplasm was drawn out into projections which resembled pseudopodia though no actual movement was observed.

In the prophase of the last spermatogonial division the chromosomes appear as the rod-like bodies shown in Fig. 83. In polar views 25 chromosomes can be distinguished, but the sex-chromosome cannot yet be singled out. Figs. 84 and 85 depict spermatogonial polar views. Figs. 86 to 90 inclusive picture various stages of the last spermatogonial division. Fig. 91 shows how the chromatin forms masses, each of which in turn makes a leptotene thread.

No detailed study of the growth period was made and consequently the nature of synapsis cannot be set forth. However, in the aceto-carmine smears made for hasty observations, the spireme was much plainer than in the sectioned material and in several cells, all the loops could be followed proving that the spireme was more or less continuous. In one cell in particular the spireme was composed of two parallel threads which were looped and twisted side by side. Figs. 92, 93, and 94 are reproductions from the aceto-carmine smears. Fig. 95 is a drawing

from fresh material in which the loops of the spireme could be made out. Quadripartite bodies similar to those of *Sympetrum* are formed from segments of the spireme. At first the longitudinal arms of these quadripartite crosses are longer than the transverse arms; but after the formation of the spindle, the four arms are nearly equal in length and the central part is less dense than the arms.

In a polar view of the primary spermatocyte division, 12 chromosomes can always be counted. These are arranged in an irregular ring of 8, surrounding four central chromosomes. More than 200 polar views were observed in which this number was present. Twelve was, therefore, thought to be the reduced number until the behavior of the chromosomes in this division was ascertained. It was then discovered that one chromosome, presumably the sex-chromosome, in nearly all cells goes over in advance of the autosomes to one pole. In consequence of such behavior, a polar view in which this chromosome is visible in the metaphase is difficult to find. Fig. 96, however, shows 13 chromosomes in a polar view and this is undoubtedly the correct number for the primary spermatocyte. Fig. 97, taken from a smear, shows only 12 chromosomes. Figs. 98 and 99 are drawn from the same cell at two different focal levels and in 99 the sex-chromosome appears above the autosomes. Figs. 100 to 103 inclusive, represent side-views of spindles with the sex-chromosome in advance of the autosomes. Fig. 104 is from an aceto-carmine smear and does not show the sex-chromosome. Figs. 105 to 109 are of especial value as they are drawn from living unstained tissue teased out in salt solution. The chromosomes could be distinguished from the protoplasm and the spindle fibers by the way in which they refracted the light. All stages in division could be found and one cell was watched while it underwent the change from the metaphase to the telophase stage, where further division stopped due probably to the fact that the solution was not absolutely isotonic with the cell protoplasm. Figs. 110, 111, and 112 are telophase stages of this division. Fig. 113 is the same stage drawn from a smear preparation.

In the secondary spermatogonial division two classes of cells

are present; one containing 12 chromosomes and the other 13 chromosomes. Figs. 114, 115, 116, are polar views of secondary spermatocytes. Fig. 116 shows two cells formed from one primary spermatocyte lying side by side, and in one there are 13 chromosomes and in the other 12. In one cell the sex-chromosome seemed to pass undivided to one pole as shown in Fig. 117 and in Fig. 118 it remains undivided at the metaphase. Figs. 119 and 120 represent secondary spermatocyte spindles while in Figs. 121 and 122 the dividing sex-chromosome stands out distinctly from the autosomes. Fig. 123 is a telophase stage.

After the second maturation division the chromosomes become fused into several large masses connected by a reticulum as in *Sympetrum*. The irregularity of the grouping leads to the conclusion that the number of masses has nothing to do with the presence or absence of the sex-chromosome and the difference in the amount of chromatin is not noticeable in the spermatids. The cytoplasm around the nucleus elongates and the centrosome is found near the largest mass of chromatin. The axial filament grows out from the centrosome which increases in size until it forms a knob. This is the only thing comparable to a middle-piece, and as the nucleus elongates it becomes so closely associated with the large chromatin mass that it can no longer be distinguished from it. Figs. 124 to 128 inclusive are drawn from living material. The spermatozoön moved with a peculiarly spiral motion such as that described for *Sympetrum*. In Fig. 127 the cytoplasm is spread out at the base of the head, probably an abnormal condition. The spermatozoön did not move rapidly but progressed continuously. In the mature spermatozoön no head-spine was visible upon the rod-like head which comprises about one third of the length of the whole spermatozoön.

VI. Historical Review.

There has been little work done upon the cytology of the Odonata. Of historical interest only are the papers by C. Th. von Siebold (1840) and Bütschli in 1871. Von Siebold deals mostly with the mating habits of the Libellulidæ, though he made some observations upon the spermatozoa. According to his account, the spermatozoa have in general the characteristic

elongated form of the insect spermatozoa and may be divided into two classes:

1. In the genera *Agrion*, *Æschna* and *Diastatomma*, they are fine, capilliform and extremely motile.

2. In the genus *Libellula* he affirms they are more solid and rod-like, and remain inactive in the male and even in the female after fertilization.

In all genera, the spermatozoa develop in the testis in bundles surrounded by delicate sheaths. In *Æschna ocellata* these bundles are so large that they can be recognized with the naked eye as white dots in the testis. In group (I) the bundles are round or oval and somewhat compressed. Before the maturation of the spermatozoa in the cyst surrounded by a sheath, there is a big vesicular area which enlarges and becomes finely granular. This is the beginning of the formation of the spermatozoa which arise within the bladder-like area of the cyst.

Von Siebold was uncertain as to the immobility of the spermatozoa in the Libellula for he says, "Ob diese Spermatozoen unter gewissen Bedingungen, welche mir entgangen sind, sich nicht dennoch bewegen solten, weiss ich nicht zu sagen."

Bütschli worked out a number of the developmental stages of the head of the spermatozoön of *Agrion puella*. He described the nucleus as sending forth a small elongation which increases in length and forms an opaque head-spine. In an immature spermatozoön, it measured 0.01 to 0.045 mm. in length while in the ripe spermatozoön it was reduced to .0078 to .009 mm. He thought that there must be some deception in his results, but in *Sympetrum* the same thing occurs.

Lefevre and McGill (1908) extended and corrected the earlier paper (1904) of McGill on *Anax junius*. The spermatogonial number was found to be twenty-seven. The small or m chromosomes of the spermatogonium divided at both mitoses and was distinct from the accessory which was a larger chromosome in the spermatogonial group. A condensed chromosome-like body persisting through the various growth stages was identified as the odd or heterotropic chromosome of the maturation division. They also found evidence which suggested that the synapsis might be a side to side union of the threads instead of end to end as first described.

In the formation of the tetrad, they found that the long axis of the tetrad is identical with the long axis of the chromatin threads of the growth period, and the first maturation division would separate univalent chromosomes and be a reducing division if the conjugation took place end to end. To quote the rest of the conclusion, "If, however, it should prove true of this form that a parallel conjugation occurs, as has been suggested, the first division would still be a reducing one, since the axis of the crosses are not reversed by the drawing out of the transverse arms and the attachment to the spindle fibers is at the end of the longitudinal arms."

Zeleny and Faust ('15) in the figures of Lefevre and McGill ('08) have measured the chromosomes and calculated a bimodal curve for the two classes of spermatozoa which must result from the behavior of the odd chromosome. "These give expected ratios of 1.00 : 1.07 on the basis of complete fusion of chromosomes and production of spermatozoa of like shape and 1.00 : 1.09 for end to end fusion of chromosomes." The spermatozoa of *Æschna canadensis* in which the chromosomal content is unknown give a bimodal but unequal curve. "The two modes are at 50.2 μ and 51.6 μ, giving a ratio of 1.00 : 1.03. This is considerably less than the ratio 1.00 : 1.07 or 1.00 : 1.09 that would be expected for *Anax junius*."

VII. General Considerations.

In *Anax*, *Sympetrum* and *Libellula*, the spermatogonial chromosomes are 27, 25, and 25 respectively. Throughout the growth period, a condensed chromatin body persists which from its subsequent behavior can be identified as the sex-chromosome. In the spermatocyte, a synapsis occurs and the autosomes form quadripartite bodies, all of which are bivalent. In the first division in *Anax* and *Sympetrum* the bivalents and the sex-chromosomes divide, and this division as far as the former are concerned probably represents a pulling apart of the univalent chromosomes which conjugated in synapsis and is therefore a true reduction division. Upon this assumption, the second division which splits the univalents must be equational. The sex-chromosome undivided goes over to one pole in the second

division. In *Libellula*, upon assuming parasynapsis, the first division represents a true reduction in every way for the bivalents divide and the sex-chromosome goes to one pole undivided. Certain stages enumerated in the description of *Sympetrum* differ from what Lefevre and McGill described for *Anax*.

VIII. Summary.

1. The maturing sex-cells are arranged in cysts in the testes, but there is no definite seriation as to age like that found in many insects and vertebrates.

2. The spermatogonial chromosomes are twenty-five in number and are closely crowded together, making it impossible to tell much about their behavior.

3. The evidence obtained seems to indicate that the leptotene threads unite side by side (parasynapsis) to form a spireme which is twisted in such a way that the loops are oriented toward one side of the nucleus.

4. This spireme breaks up into segments which open out presumably along the original axis of synapsis to form rings. These condense into crosses and then into quadripartite bodies or prophase chromosomes.

5. The primary spermatocyte contains 12 bivalent autosomes and one sex-chromosome. The bivalents divide apparently along the line of their original junction making this the reduction division for them while the sex-chromosome divides equationally.

6. In the second spermatocyte division all the univalent autosomes divide equally while the sex-chromosome passes to one pole undivided; thus two kinds of spermatids are formed. These change into linear spermatozoa which show no visible difference. But the one which possesses the sex chromosome must be physiologically different.

7. In *Libellula basalis* the spermatogonial number is 25; the reduced number, 13, consists of 12 bivalent autosomes and 1 univalent sex-chromosome. The sex-chromosome, unlike its procedure in *Sympetrum*, passes undivided to one pole in the primary spermatocyte division, forming two kinds of secondary spermatocytes. In the secondary division the sex-chromosome divides equally. Two kinds of spermatozoa are thus formed which must have a functional difference.

BIBLIOGRAPHY.

Davis, H. S.

'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. Mus. Comp. Zool. Harvard, Vol. 52, No. 2.

Janssens, F. A.

'09 La théorie de la chiasma-typie. Nouvelle interprétation des cinêses de maturation. La Cellule, Tom. 25.

Lefevre and McGill.

'08 The Chromosomes of Anasa tristis and Anax junius. Amer. Jour. Anat., Vol. 7, No. 4.

Lewis, R. L. and W. H.

'15 Mitochondria (and Other Cytoplasmic Structures) in Tissue Cultures. Amer. Jour. of Anat., Vol. 17, No. 3.

McClung, C. E.

'02b The Spermatocyte Divisions of the Locustidæ. Kansas Univ. Sci. Bull., 14.

'14 A Comparative Study of the Chromosomes in Orthopteran Spermatogenesis. Jour. Morph., Vol. 25, No. 4.

McGill, C.

'04 The Spermatogenesis of Anax junius. The Univ. of Missouri Studies, Vol. 2, No. 5.

Schriner, A. und K. E.

'04 Die Reifungsteilungen bei den Wirbeltieren. Anat. Anz., 24.

'05 Ueber die Entwicklung der männlichen Geschlechtszellen von Myxine glutinosa (L). Arch. de Biol., Bd. 21.

'06a Neue studien über chromatinreifung der Geschlechtszellen. I. Die Reifung der männlichen Geschlechtszellen von Tomopteris onisciformis. Arch. Biol., Bd. 22.

'06b II. Reifung der männlichen Geschlechtszellen von Salamandra maculosa, Spinax niger und Myxine glutinosa.

'08 Gibt es eine parallelle Konjugation der chromosomen? Erwiderung an die Herren Fick, Goldschmidt und Meves Videnskbs. Selskab Skrifter. I. Math., Natura Klasse, No. 4.

v. Siebold, C. Th.

'41 Ueber die Fortpflanzungsweise der Libellulinen. Germar, Zeitschrift. f. die Entomologie, Bd. 1 und 2.

Sutton, W. S.

'00 The Spermatogonial Divisions in Brachystola magna. Kansas Univ. Quart., Vol. 9.

'02 On the Morphology of the Chromosome Group in Brachystola magna. Biol. Bull., Vol. 4, No. 1.

Wilson, E. B.

'12 Studies on Chromosomes. VIII. Observations on the maturation phenomena in certain hemiptera and other forms, with considerations on synapsis and reduction. Jour. Exp. Zool., Vol. 13, No. 3.

Zeleny, C., and Faust, E. C.

'15 Size Dimorphism in the Spermatozoa from Single Testes. Jour. Exp. Zool., Vol. 18, No. 2.

EXPLANATION OF PLATES.

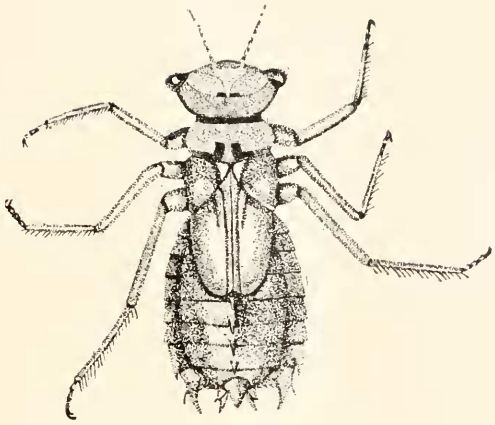
Unless otherwise indicated the figures are drawn at a magnification of 2,700 diameters. The drawings of the fresh material are made with a magnification of 1,500 diameters. Fig. 24 is magnified 3,900 diameters. Plate I. is reduced one-third while Plates II. to VI. inclusive are reduced one-fifth. The nuclei drawn are usually from sections not directly through the center of the cells, as they contain less chromatin and are consequently clearer.

PLATE I.

Sympetrum semicinctum (Say).

FIG. 1. A drawing of the nymph of *Sympetrum semicinctum* (Say).

FIG. 2. A cross-section of the testis of a nymph.



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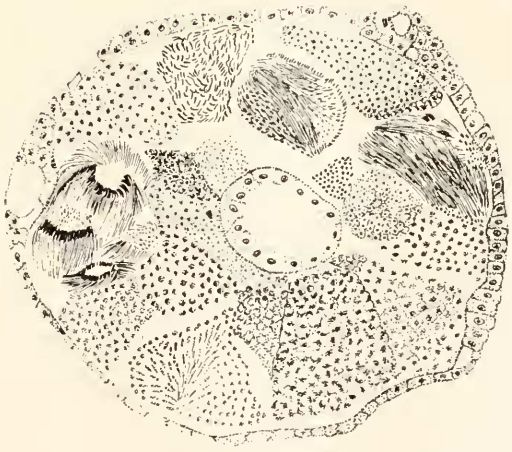

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Plate II.

Sympetrum semicinctum (Say).

Fig. 3. Part of a cyst showing degenerating spermatogonia.

Fig. 4. A group of spermatogonial cells.

Fig. 5. A spermatogonial cell from a smear preparation.

Fig. 6. Polar view of spermatogonial chromosomes from an aceto-carmine smear.

Fig. 7. Polar view of metaphase of spermatogonial nucleus in which only a few chromosomes are in focus.

Fig. 8. Polar view of metaphase of spermatogonial nucleus.

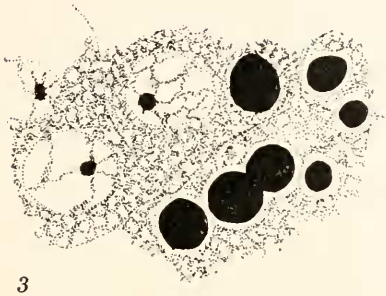
Figs. 9, 10, and 11. Telophases of spermatogonial divisions.

Figs. 12 and 13. Polar views of telophases of last spermatogonial division.

Figs. 14, 15, and 16. Spermatogonia with the round, dense body that may correspond to the mass in the growth period which subsequently becomes the sex-chromosome.

Fig. 17. Spermatogonia in the diffuse period before the growth changes begin.

Figs. 18, 19, and 20. Massive bodies in the nuclei at the beginning of growth period. One mass which later forms the sex-chromosome is much darker and more compact and is marked *X*.



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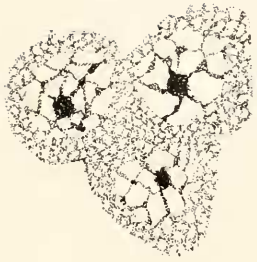

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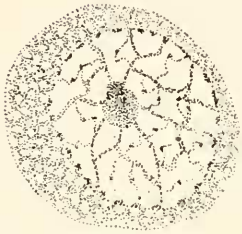

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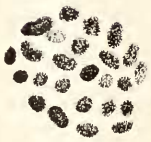

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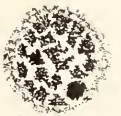

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Plate III.

Sympterum semicinctum (Say).

Figs. 21, 22 and 23. Leptotene threads forming from the masses. In 22 and 23 the sex-chromosome is present.

Figs. 24 (× 3,900), 25 and 26. Synaptic stages in which parallel leptotene threads unite.

Figs. 27, 28 and 29. Synizesis stages.

Figs. 30 and 31. Polar views of cut threads of a stage like Fig. 29.

Figs. 32 and 33. Chiasmas.

Fig. 34. Threads opened out into apparent eights.

Fig. 35. A true eight.

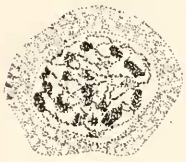
Fig. 36. A cell of *Anax junius* which shows chromomeres in the segments.

Fig. 37. Cell with signet-ring loop.

Fig. 38. Signet-ring loop from several angles.

Figs. 39, 40 and 41. Contain prophase crosses. Fig. 40 shows the sex-chromosome and Fig. 41 contains the largest cross.

Figs. 42, 43 and 44. Polar views of the chromosomes of the primary spermatocyte division.



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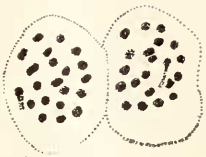

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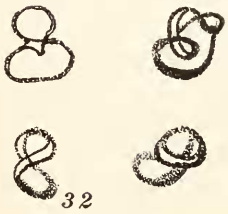

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PLATE IV.

Sympetrum semicintum (Say).

FIGS. 45, 46, AND 47. Side views of metaphase of primary spermatocyte division.

FIG. 48. Anaphase of primary spermatocyte division.

FIGS. 49, 50, 51 AND 52. Telophases of primary division.

FIG. 53. Polar view of telophase of primary division which shows the sex-chromosome at one side.

FIGS. 54, 55, 56 AND 57. Polar view of chromosomes of secondary spermatocyte.

FIGS. 58, 59, 60, 61 AND 62. Metaphase views of secondary spermatocyte divisions with the sex-chromosome in various positions.

FIGS. 63. 64. Telophases of secondary spermatocyte division.

FIG. 65. Metaphase of secondary spermatocyte division from aceto-carmine smear.

FIGS. 66 AND 67. Polar view of telophase of secondary spermatocyte division showing sex-chromosome.

FIGS. 68, 69 AND 70. Spermatids.

FIG. 71. Spermatid showing tail filament.

FIGS. 72 AND 73. Spermatids which have elongated and formed head spines.

FIG. 74. A sperm head from a smear preparation.

FIGS. 75 AND 76. Cross-section of spermatozoa to show the chromatin condensed around the nuclear wall.

FIGS. 77, 78 AND 79. Spermatozoa from aceto-carmine preparations.

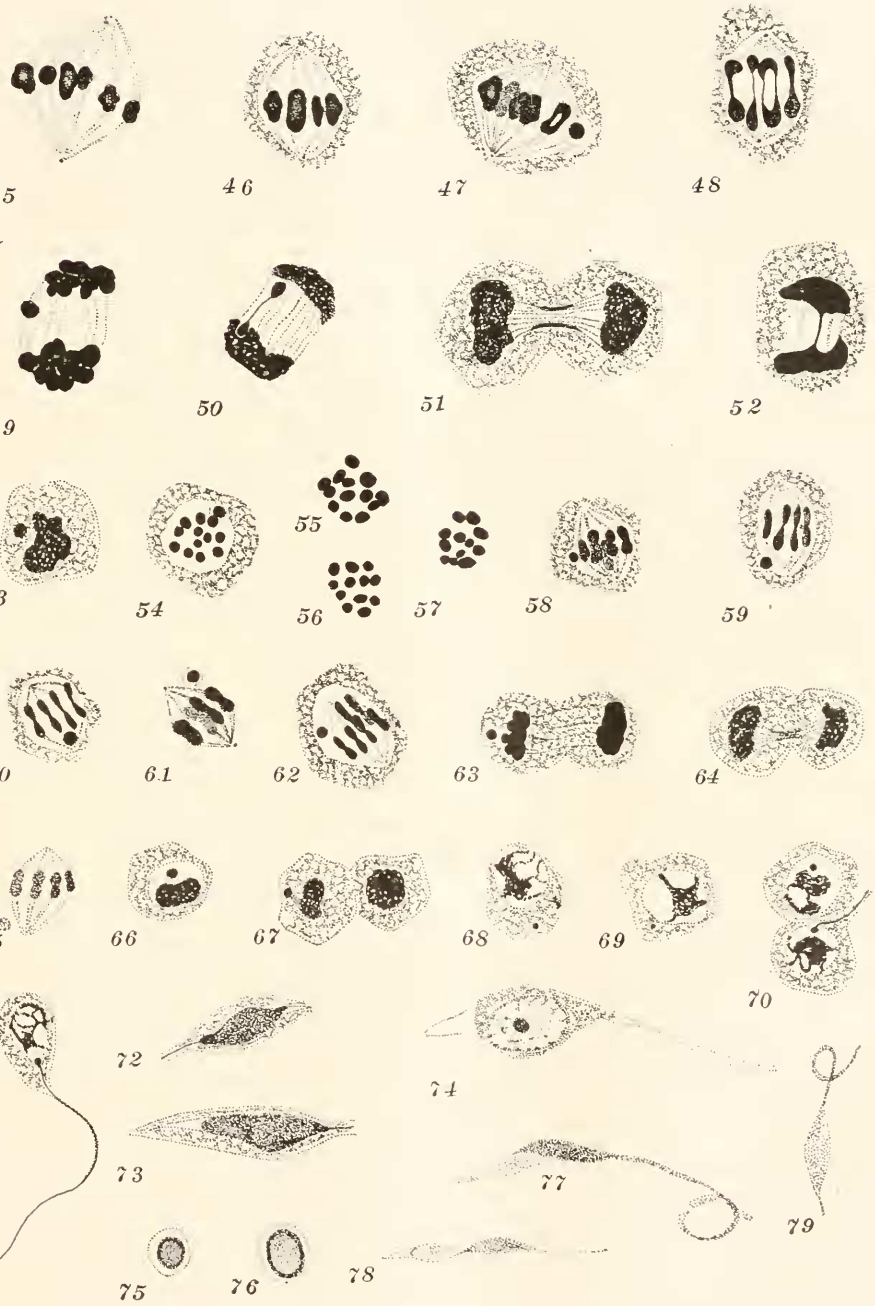
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Plate V.

Libellula basalis.

Fig. 80. Spermatogonium from aceto-carmine preparation.

Fig. 81. Spermatogonium from iron-hematoxylin section.

Fig. 82. Spermatogonium from fresh material (× 1,500).

Fig. 83. Prophase chromosomes of spermatogonium.

Figs. 84 and 85. Polar view of chromosomes of last spermatogonial division.

Figs. 86–90. Spermatogonial division stages.

Fig. 91. Formation of leptotene threads.

Figs. 92, 93 and 94. Spireme stages from aceto-carmine preparations.

Fig. 95. Spireme stage from fresh material.

Figs. 96, 97, 98 and 99. Polar views of chromosomes of primary spermatocyte division. Fig. 97 is from an aceto-carmine preparation and shows only 12 chromosomes. Figs. 98 and 99 are taken from the same cell, at different focal levels.

Figs. 100, 101, 102 and 103. Various stages in primary spermatocyte division showing the sex-chromosome going to one pole undivided.

Fig. 104. Metaphase of primary spermatocyte division from an aceto-carmine preparation.

Figs. 105 and 106. Primary spermatocyte stages (× 1,500) from fresh material.

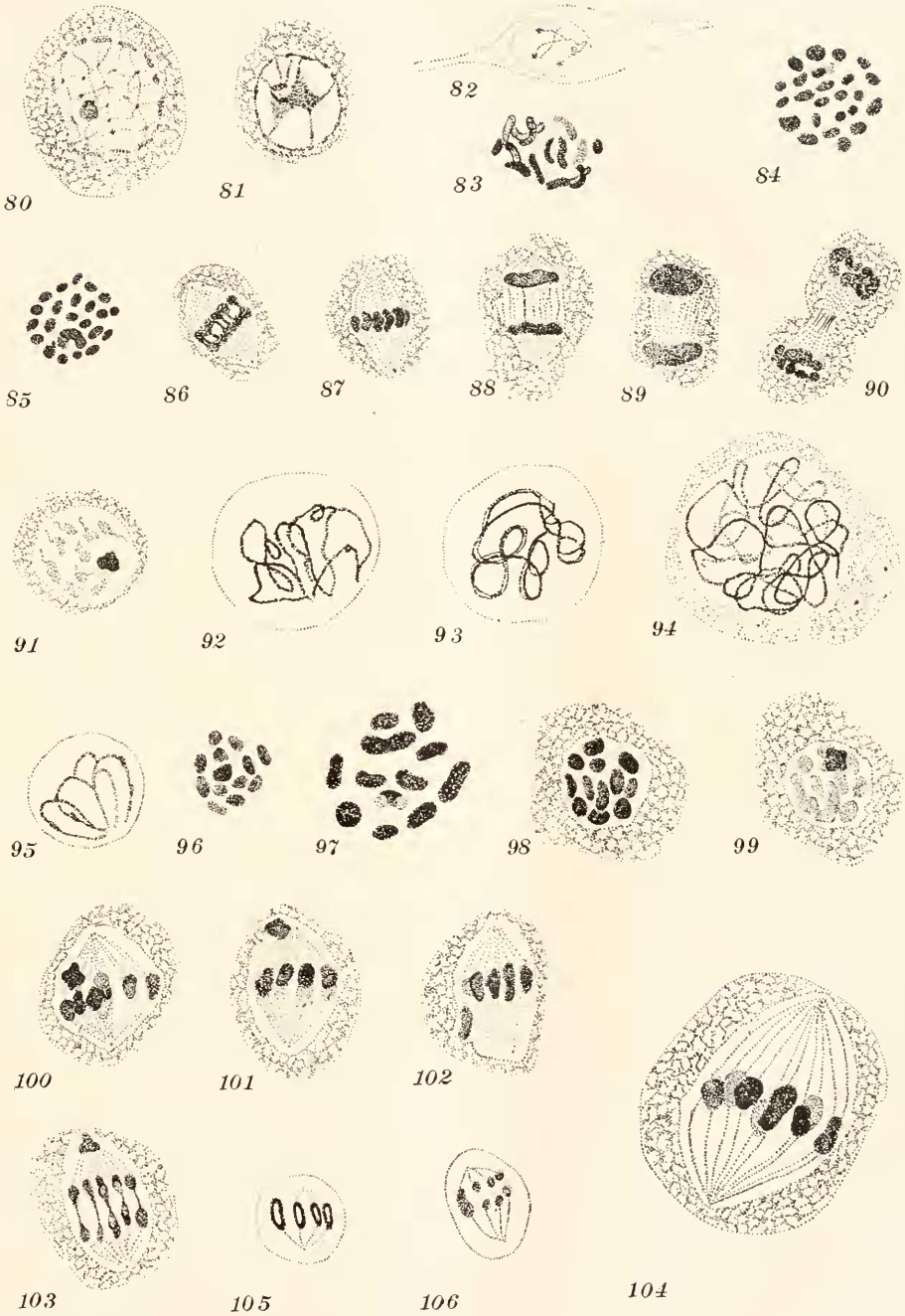

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PLATE VI.

Libellula basalis.

FIGS. 107, 108 AND 109. Primary spermatocyte stages (× 1,500) from fresh material.

FIGS. 110, 111, AND 112. Telophases of primary spermatocyte division.

FIG. 113. Telophase of primary spermatocyte division from aceto-carmine smear.

FIGS. 114, 115, AND 116. Polar views of chromosomes of secondary spermatocytes.

FIG. 117. One chromosome apparently going to one pole undivided in secondary spermatocyte division.

FIG. 118. Sex-chromosome lagging behind in secondary spermatocyte division.

FIGS. 119, 120, 121, 122, AND 123. Various stages in secondary spermatocyte division.

FIGS. 124–128 INCLUSIVE. Spermatozoa drawn from living material.


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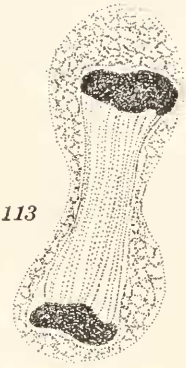
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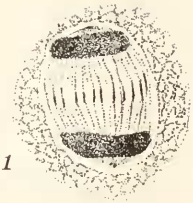
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BIOLOGICAL BULLETIN

ON THE BEHAVIOR OF AMEBA TOWARD FRAGMENTS OF GLASS AND CARBON AND OTHER INDIGESTIBLE SUBSTANCES, AND TOWARD SOME VERY SOLUBLE SUBSTANCES.

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Zoölogical Laboratory, University of Tennessee.

Contents.

Introduction.

The experiments recorded in this paper were carried out in order to come a step closer to understanding the nature of the stimulus which, emanating from an insoluble particle such as ovalbumin, zein, or lactalbumin, results in an ameba moving

toward that particle. As pointed out in a previous paper (Schaeffer, '16b) in which the reactions of ameba toward proteins are recorded, these isolated proteins, although insoluble enough to satisfy the practical chemist that they may be classed as 'insoluble,' may nevertheless undergo an inappreciable amount of solution sufficient to stimulate an ameba's sense organs. There is however no direct means of knowing that such solution takes place; it is nothing more than a possibility. It has seemed practicable therefore to let the investigation of the behavior toward isolated *insoluble* proteins stand for the present, and to test the reactions of ameba toward some of the purest and most insoluble substances known to determine whether it is necessary that a substance be soluble in order that an ameba may sense it at a distance. In addition to these tests, a number of experiments were also made with very soluble substances and with solutions.

The main conclusions of this paper are based on the reactions toward carbon, glass, tyrosin and peptone. A sufficient number of experiments with these substances are described and figured, I trust, to illustrate, if not to prove, the conclusions. But in addition to these, one or more typical experiments with each one of various other substances have been figured to support the conclusion that the behavior toward the substances specified above is not directly dependent upon the chemical nature of the substances, but upon their more generalized physical properties.

Reactions to Chemically Insoluble Indigestible Substances.

Carbon.—This substance was prepared as follows. India ink in stick form was boiled in xylol until the xylol remained clear. The residue was washed in chloroform and then boiled in sulphuric acid, then filtered and washed with distilled water. The residue was then boiled in potassium hydroxide solution. After acidifying, the carbon was filtered and washed with distilled water acidified with hydrochloric acid. The carbon was then dried and heated to redness for fifteen minutes in a closed platinum crucible. This method of purification should have removed all the soluble constituents present with the carbon in the india ink.

Although carbon as thus prepared is quite insoluble, it is not inert, for carbon has the property of adsorbing certain gases from the surrounding medium. In order to render this action as ineffective as possible on the sense organs of the ameba, the carbon grains, which it will be remembered were in all cases microscopic, were immersed in the water in which the ameba was, thirty minutes before being brought near the ameba. It is believed that this period of immersion permitted the adsorptive and diffusive processes to come as near as is possible to an equilibrium.

A grain of pure carbon was placed in the path of a granular[1] ameba (Fig. 42, Plate I.). As the ameba moved forward it turned to the right, but after passing the carbon it turned to the left. After coming into contact with the carbon a pseudopod was thrown out on the right through which the ameba moved away. The same piece of carbon was again laid before the ameba—47—but the behavior was indefinite.

Several experiments were made on a granular ameba from another culture. A grain of carbon which was laid in its path produced a mild positive reaction: the partial encircling of the carbon—183–188. The carbon grain was then shifted but the commotion caused by moving it led the ameba to react negatively. The carbon was shifted again, producing finally a mild positive reaction. When the carbon was shifted again—189—the ameba turned to the right. As it passed by the carbon, at about twenty microns, two little pseudopods were sent out in the region of the carbon, one of which was headed directly toward the carbon. The ameba moved directly into contact with the carbon, and then moved on through that pseudopod—193.

In the path of another granular ameba was placed a grain of carbon—250. The ameba moved on straight forward, passing the carbon on the right. When the ameba was about half past the carbon numerous pseudopods began to make their appearance on both sides of the ameba, before the ameba had come into

[1] See my paper ('16) pp. 533–536, where the granular and raptorial forms of amebas are described. After this paper was in manuscript the specific identity of the amebas was specially examined. The 'granular' amebas were of two species: *Amœba proteus* and *A. discoides*. The 'raptorial' were of the species *A. dubia*. See my paper in Science, '16, vol. 44, pp. 468–469.

contact with the carbon. The ameba came into contact with the carbon later but the direction of movement was not on that account changed. Although the stimulus from the carbon grain was localized yet the resulting change in behavior involved the whole ameba, for it broke up into a number of pseudopods of which those on the right side of the ameba had of course no direct relation whatever with the carbon grain. The *meaning* of these pseudopods on the right is obscure, though pseudopods are frequently formed in this way under such conditions as surrounded this experiment. Orderly movement in clavate form was disturbed by the sensing of the carbon grain, but after the carbon grain was left behind, orderly movement was again resumed. It should be noted especially that a pseudopod was formed on the right side directly opposite the carbon. This phenomenon is frequently observed in ameban behavior. A number of cases are described in a previous paper (Schaeffer, '16). See also Fig. 224 in this paper. Throughout the whole experiment the general direction of motion was not changed, but it could not be predicted from Fig. 259 in what direction the ameba was then going to move. This experiment is a very good example of the phenomenon of functional inertia (Schaeffer, '12, '14)—the tendency in an ameba to continue moving in the direction in which it started to move—about which I shall have more to say later.

A grain of carbon was placed in the path of a raptorial (see footnote, p. 305) ameba—216. The ameba moved into contact with the carbon, then forked and moved on through the right prong. The carbon grain was then shifted so that it lay to the right of the ameba's path—220. The ameba moved forward, turning away from the carbon at first, but later a side pseudopod was sent out directly toward it. When the ameba was about forty microns from the carbon another pseudopod, further anterior, was also sent out toward the carbon. The posterior pseudopod was the first one to come into contact with the carbon, and as it continued moving forward, it pushed the carbon grain along. The anterior pseudopod moved into contact with the carbon. A pseudopod directly opposite the anterior one enlarged and carried the ameba away. Both pseudopods extended toward

the carbon were retracted as the ameba moved away through the pseudopod which had been forming on the opposite side. Some time later another grain of carbon was laid in the ameba's path—226. Two pseudopods were formed on the right, through the anterior one of which the ameba moved away, disturbed probably by the commotion caused by placing the carbon in the ameba's path. The same piece of carbon was again laid ahead of the ameba—230. The ameba at first turned away from the carbon—231, 232—but later turned toward it—233. A pseudopod was then sent out toward the carbon until it came into contact with the carbon—235–237, then it was retracted and the ameba moved off, directly away from the carbon—239. A few minutes later a new piece of carbon was laid in the ameba's path some distance ahead—240. The ameba then broke up into several pseudopods, but finally moved ahead a short distance through the main pseudopod. A pseudopod was then sent out on the right, which moved into contact with the test substance and apparently ingested it in a typical food cup. The ameba kept on moving forward, and two minutes after apparent ingestion the carbon was left behind. (The curved leader line straightened out, is the measure of the distance the ameba moved away in a straight course from the carbon grain.) The same piece of carbon was again laid ahead of the ameba—264. As the ameba moved forward it turned to the left and away from the carbon, but as it passed by the carbon two pseudopods were sent out toward it—267. The posterior one came into contact with the carbon, moved on over it, and spread out; but no attempt at ingestion was made. The pseudopod forked and the ameba moved along the left prong. A new piece of carbon was then laid to the left of the ameba's path—271. As the ameba moved forward, a pair of side pseudopods which were begun simultaneously on opposite sides—274—continued to enlarge until the one on the left began to flow over the carbon. Then the pseudopod on the right was retracted and the ameba flowed away through the pseudopod extended over the carbon.

To summarize the behavior toward carbon: The most striking feature of the behavior toward pure carbon is that the ameba can sense this substance at a distance of at least forty microns. It

is of course not surprising that soluble objects should be thus sensed, but the sensing of an absolutely insoluble substance at a distance is unique among eyeless animals. It is possible that the carbon grains acted as permanent centers of diffusion of gases adsorbed previously, or adsorption of gases dissolved in the water, and so may have produced differences in their distribution in the water. If these differences in distribution of gases are assumed to come within sensing range of the ameba, then one could understand the observed behavior. I believe however that the gas adsorptive qualities of carbon do not in themselves constitute the stimuli to which ameba reacts when it comes near the carbon; for glass, which is not supposed to adsorb gases to the same extent as carbon, stimulates ameba in a similar manner and quite as markedly.

Practically all of the behavior toward carbon is positive. The negative behavior observed was due to the commotion produced by placing the carbon in position. In most cases the pseudopod which was sent out to the source of the stimulus, was retracted after it had come into contact with the carbon, but in some cases the ameba flowed on through such an exploring pseudopod. Only in one case was ingestion attempted. That it is a real case of partial ingestion is shown by the fact that the process was incomplete; for if the initial stimulus had come from an unobserved small flagellate, for example, on the carbon grain, it is fairly certain that the ingesting process would have been completed. It is reasonable to suppose that the stimuli causing partial ingestion came from the carbon grain.

Both granular and raptorial amebas react to carbon at a distance. The raptorial seem to be attracted somewhat more strongly than the granular.

Glass.—Although glass is a complex substance and is very slightly soluble, neither of these properties by themselves play a part in the stimuli received by amebas; for the fragments of glass were taken, in nearly every case, from the dish or slide on which they and the amebas were later placed in experimenting. The effect of its solubility may therefore be thought to have been cancelled physiologically by the solubility of the glass surface on which it lay.

The glass dishes and the fragments were all carefully cleaned before using. The glass fragments were powdered in a glass mortar and then washed. The culture fluid was carefully filtered, and the amebas were transferred through several washes of filtered culture solution.

A fragment of glass was placed near a raptorial ameba—125. As the main pseudopod moved forward it forked, the left limb moving toward the glass—126, 127. The right pseudopod moved on for a short distance, then turned sharply toward the left and moved into contact with the glass—128–130. The right limb was then withdrawn and the ameba moved off through the left. The glass seems to have been sensed at a distance of about forty microns—128, 129—perhaps at sixty microns—126. A few minutes later a new fragment of glass was laid in the ameba's path—133. The ameba at first turned to the right, but a little later a pseudopod was sent out on the left which moved almost into contact with the glass, but was then withdrawn—136–139. The main pseudopod broke up into three pseudopods, one of which moved a short distance toward the glass and was then retracted. The pseudopod on the right, which with the left one already mentioned formed a pair of opposite pseudopods, then became the main pseudopod through which the ameba moved away. There is no doubt that the ameba received stimuli proceeding from the glass; the formation of the pair of opposite pseudopods shows it, as does also the retraction of the left pseudopod before the glass was reached. The same piece of glass was again laid in the ameba's path—141. The ameba moved toward it a short distance, then turned slightly to the right and moved on—142–144. When the tip of the main pseudopod was even with the glass fragment, a side pseudopod was sent out toward the glass and into contact with it—145, 146. The tip of the main pseudopod also turned over toward the glass and then moved into contact with it. Both pseudopods were then withdrawn while the ameba moved off through a pseudopod on the right. The ameba sensed the glass in Fig. 142 at a distance of over sixty microns. The same piece of glass was then shifted —149. The ameba moved directly forward to within about forty microns of the glass, when the tip of the pseudopod spread

out and later forked and flowed on under the glass fragment. The same piece of glass was again laid before the ameba, but the behavior does not show definitely that the glass was sensed at a distance. The same piece of glass was then shifted and again laid to the left of the ameba's path—160. The ameba moved forward a short distance, then sent out a pseudopod on the left directly toward the glass—162. The pseudopod was called forth doubtless by the agitation of the needle in placing the glass in position, for hungry raptorial amebas are readily thus stimulated. But the glass was actually sensed in Figs. 163 and 164, for the pseudopod directed slightly to the right of the glass turned so as to go directly toward the glass. After coming into contact with the glass a pseudopod was formed on the convex side of the main pseudopod, a region especially favorable to the formation of new pseudopods, through which the ameba moved away. A new piece of glass was then laid before the ameba—168. A pseudopod was thrown out on the right through which the ameba moved on—169–171. This pseudopod turned strongly to the left toward the glass. When about eighty microns from the glass—172—a pair of opposite pseudopods were formed near the tip of the main pseudopod. The left member of this pair of pseudopods moved directly toward the glass. When almost in contact with the glass this pseudopod was retracted—173. The ameba moved away through the main pseudopod. The same piece of glass was again laid before the ameba—175. The ameba moved toward it a short distance, when a pair of opposite pseudopods were formed near the tip of the main pseudopod—177, 178. As the ameba moved past the glass, another pair of opposite pseudopods were formed near the tip of the main pseudopod—178. Neither of these pseudopods moved far before they were retracted—179, 180. Before the ameba moved out of sensing distance of the glass, it turned strongly to the left and encircled the glass through 180° at a distance of about sixty microns. Two pseudopods, soon to be retracted, were formed on the convex side of the ameba during the latter stage of the encircling reaction—181, 182.

A small fragment of glass was laid in the path of a granular ameba—194. The ameba broke up into several pseudopods, of

which the left member of the middle pair became the main pseudopod. As this one moved forward with the glass particle on its right, a pseudopod which appeared on its right, enlarged and moved directly into contact with the glass—197, 198. When the pseudopod came into contact with the glass, streaming became more rapid and the ameba flowed over the glass particle and moved away.

To summarize: The behavior of ameba toward glass fragments, under the conditions above outlined, demonstrates even more clearly than the reactions toward pure carbon, that insoluble objects can be sensed at a distance. The maximum distance at which glass can be sensed, as demonstrated by experiment, is about sixty microns, though it is probable that in several of the experiments the amebas sensed the glass particles at 100 microns. The ameba does not always react positively when glass is sensed, but positive behavior is much more frequent than negative. Although the ameba starts moving toward the glass particle in almost all cases, it sometimes reverses the direction of motion when almost in contact with it. In most cases however the ameba continues moving until it comes into contact with the glass, and then the behavior becomes more or less indifferent. No attempt was made to eat particles of glass.

Graphite.—A grain of Merck's purified graphite was laid in the path of a granular ameba—202. The ameba turned to the right and moved directly toward the graphite until it came within about fifteen microns of the object, when protoplasmic streaming was interrupted for an instant and then directed upwards and away from the graphite—207–209. The piece of graphite was then shifted—210. The ameba turned to the right and away from the graphite, but a pseudopod which was then sent out on the convex side elongated and became the main pseudopod until it came into contact with the graphite (the contact stage is not figured)—214, 215. It was then slowly retracted while the previous main pseudopod became active again and led the ameba away. The precision of the reaction indicates that the graphite was sensed at a distance of at least sixty microns.

The effect of graphite on the reactions of ameba is similar to that produced by glass. Graphite usually produces a positive

reaction. In no case was ingestion attempted. The solubility of graphite was not tested by me.

Silicic Acid.—Merck's Pure, by Wet Process. A small grain of silicic acid was laid in the path of a granular ameba—309. The ameba moved forward a short distance—within about forty microns of the silicic acid—then swelled up at the anterior end and finally sent out a pseudopod on the left through which the ameba moved off. A new grain of silicic acid was then encountered—314. The ameba moved directly into contact with it at the side. The ameba then moved off through a pseudopod sent out posterior to the test object—319–321. A new grain of silicic acid was then laid in the ameba's path—322. After moving toward it a short distance—until within about thirty-five microns of the acids—323, 324—the ameba moved away through a pseudopod thrown out on the left. Another new grain of silicic acid was then laid in front of the ameba—328. The ameba moved forward to within sixty-five microns of the acid, then moved off through a pseudopod thrown out on the left.

Silicic acid is sensed at a distance like carbon, glass and graphite, but with the ameba used in the experiments recorded above, the behavior was nearly always negative. Owing to incomplete knowledge concerning the purity of this substance, it is not clear what the meaning is of the preponderance of negative behavior in the reactions of this ameba. The negative tendency cannot have been due to lack of hunger however for a grain of globulin was readily eaten a few minutes later.

Hematin.—Merck's, according to Nencki. This compound results from the decomposition of hæmoglobin, and is very rich in iron. The black "melanin" produced by the malarial organism is supposed to be hematin, and is said to have a toxic effect on the human body. The following experiment is typical of the behavior of ameba toward hematin. A grain of hematin was placed in the path of a granular ameba—28. As the ameba passed it on the right at a distance of about forty microns, several small pseudopods were sent out but none of them came into contact with the hematin.

Hematin seems to call forth about the same behavior as glass or carbon; perhaps the positive reactions are not quite so decided.

Hematin is not toxic to the ameba; when eaten with food materials it may remain in the ameba for many hours without producing any ill effect.

Indigotin.—Merck's reagent, indigo blue. To the left of the path of a granular ameba was placed a grain of indigotin—278. The ameba moved forward until the tip of the main pseudopod was past the test object—279. A pseudopod was then sent out to the left, which moved into contact with it—280, 281. As the ameba moved forward, it moved out of contact with the indigotin. A small pseudopod which was sent out into contact with the indigotin from the mid-region of the ameba—283—remained in contact with the test substance for a few minutes while the ameba moved on, but it was finally pulled away.

Ameba reacts more decidedly positively to indigotin than to hematin, glass, or carbon. In no case however was ingestion attempted.

Cholesterin.—Eimer and Amend's. In the path of an active granular ameba with many pseudopods, was placed a grain of cholesterin—35. As the ameba moved forward two pseudopods were sent out toward the cholesterin—37—but only one of them came into contact with it. The ameba moved off through a pseudopod thrown out on the right, leaving the cholesterin behind.

As far as my experiments go, it appears that cholesterin belongs in the same class as carbon, glass, etc. No attempt at ingestion is made. Cholesterin is sensed at a distance of at least fifty microns.

Starch Grains from Arrowroot.—Taylor's Commercial. A grain of arrowroot starch was placed in the path of a three-pronged raptorial ameba—288. The ameba moved forward through the middle pseudopod directly into contact with the starch grain and then passed on, on the right, after forming and retracting two pseudopods on the left. The ameba then happened to move toward another mass of arrowroot starch—294. When the ameba came within about thirty microns of the starch, it withdrew from the starch and moved forward to the right—296—but the pseudopod lying nearer the starch became the main pseudopod—297. When the tip of the main pseudopod

had passed beyond the starch—298—two side pseudopods were thrown out toward the starch—299—the anterior one of which came into contact with it—300. Both the side pseudopods were retracted as the ameba started to flow away through the vestige of a former pseudopod shown in Fig. 296 with the arrow. While moving forward the ameba passed another mass of starch grains without reaction. A grain of globulin was then ingested but was excreted a few minutes later.

Arrowroot starch grains are sensed at a distance and usually induce positive behavior. The reactions are more decidedly positive than those induced by glass or carbon, but no attempt at ingestion was observed. A number of experiments with corn-starch produced essentially the same results as those with arrow-root starch.

Lead Oxide.—Eimer and Amend's Pure Yellow Lead Oxide. A raptorial ameba was isolated and a small mass of lead oxide placed in its path—473. As the ameba moved forward it turned toward the oxide—475, 476—showing that this material may be sensed at a distance of at least forty microns. After the ameba came into contact with the oxide a small pseudopod was thrown out posterior to it—479. The oxide was partially surrounded and the behavior suggested the first stage of ingestion, but the ameba moved on leaving the oxide finally behind—484. A new mass of oxide was then placed before the ameba—485. The ameba moved directly forward into contact with the oxide, and there was observed again what seemed like the initial stages of ingestion—487. The ameba then broke up into several pseudo-pods—488. A food cup was formed between the two pseudopods on the left, but nothing could be observed in it. It is probable that the presence of the lead oxide was the cause of the formation of the empty food cup. The ameba finally moved on leaving the oxide behind. A piece of globulin which was then laid before the ameba remained uningested perhaps because of the just previous disagreeable effect of the lead oxide. Several essentially similar instances are recorded in my previous papers cited above. A new mass of lead oxide was then placed in the path of another granular ameba—491. The ameba moved for-ward a short distance then turned to the right—492. The

ameba then broke up into four pseudopods, two of which were directed toward the oxide—494. The ameba moved off to the right without further reaction toward the oxide.

Lead oxide induces strongly positive behavior in raptorial amebas. Not only are the amebas induced to move toward this substance, but occasionally the initial stages of ingestion seem to be called forth by it. In this respect lead oxide stands on a level with, or above, some food substances such as zein or ovalbumin. In strong contrast to the behavior of raptorial amebas toward lead oxide is that of the granular amebas. In these, negative behavior is nearly always produced by this substance. Why there should be this difference is not clear. The solubility of this substance was not tested by me.

Among other insoluble substances that were used in these experiments is iron. This metal cannot be obtained perfectly pure and it also undergoes chemical action in the water. Particles of it were agitated by means of an electromagnet beneath the stage of the microscope, but the apparatus was rather crude and no definite results were obtained.

Reactions to Very Soluble Digestible Substances.

Substances belonging to this class, such as gelatine and tyrosin, are much less satisfactory to work with than those substances that are insoluble, or only very slightly soluble; for the stimuli proceeding from very soluble substances cannot be definitely localized, and the behavior often appears uncertain. The resulting behavior is consequently difficult to interpret. Notwithstanding these objections, experiments in which very soluble substances may be used are of value in order to learn in a general way what effect the degree of solubility may have on ameba.

Tyrosin.—The product used bore Merck's guarantee of purity. A mass of tyrosin thirty microns in diameter dissolves in water in about ten minutes. A grain of tyrosin was placed in the path of a granular ameba—6. The ameba turned to the right and moved on, avoiding the tyrosin. A new grain of tyrosin was then laid in the ameba's path and negative behavior again followed—10. A third grain of tyrosin was then presented—13—and the ameba moved on past it with apparent indifference.

(A grain of globulin which was next presented was, after some uncertainty in behavior, finally ingested in a food cup pointing upwards.) A few minutes after the globulin was ingested another grain of tyrosin was laid in the ameba's path—18. The ameba moved into contact with it and then ingested it while moving on over it. No period of rest ensued. Another grain of tyrosin was then laid a little to the right of the ameba's path, but before coming quite into contact with it, the ameba moved away to the left.

This series of experiments shows very well the effect of previous behavior upon a closely following reaction. First, two trials with tyrosin produced negative behavior. The third trial resulted in indifferent behavior, doubtless because it was the third time the test substance had been encountered. The ameba was a long time in eating a grain of globulin which was next presented, and at first the ameba reacted indifferently toward it. It is more than likely that the previous experience with tyrosin developed this condition of indifference in the ameba. But this condition was entirely overcome by the reactions involved in eating the globulin, for when the next grain of tyrosin was presented, it was ingested. Thus the effect of previous behavior influenced the ameba's succeeding reactions more than the nature of the stimuli received in these reactions. But the newly created tendency to positive behavior was of short duration, for when another grain of tyrosin was presented only mild positive behavior, followed by avoidance, was observed.

A tyrosin grain was placed in the path of another granular ameba—51. Very remarkable behavior followed. The ameba moved forward into contact with it and then proceeded to flow on over it. When the anterior end lay over the tyrosin, it formed itself into an inverted shallow cup over the tyrosin—55. But no sooner was the food cup formed and ready to close up over the tyrosin than the anterior end was lifted up, away from the tyrosin, and the middle and posterior regions of the ameba contracted. The effect was of course to remove the anterior end of the ameba from the dissolved or dissolving tyrosin. The food cup was completed however and persisted in the ameba for some time. The ameba moved away from the tyrosin for a short

distance but it soon came again within sensing range of the tyrosin grain—59. The ameba became aware of the center of diffusion of the tyrosin at a distance of about 125 microns. The ameba moved toward the tyrosin grain, then over it, then formed a food cup, and later withdrew, just as in the preceding trial. The tyrosin had gone completely into solution however when the ameba withdrew. Another tyrosin grain was then laid before the ameba—64. The ameba moved forward into contact with it and then repeated, substantially, the behavior observed in the two preceding tests.

After the ameba had withdrawn a short distance from the tyrosin, and had become more or less quiet, another ameba—71—came from the opposite direction and proceeded at once to form a typical food cup over the tyrosin grain. When the food cup was nearly completed over the tyrosin, it was suddenly extended to take in part of the original ameba—73. The tyrosin became imbedded in the protoplasm while the new ameba attempted to eat the other one—74. The attempt to capture the old ameba was soon given up as this ameba became active and moved out of reach of its would-be captor—75–77.

A new grain of tyrosin was laid in the path of the ameba that had been partially captured (the ameba shown in Fig. 70)—79. The ameba turned to the left avoiding the tyrosin, but later while passing by the tyrosin, a side pseudopod was sent out toward it—84, 85. This pseudopod moved over the tyrosin, swelled out and formed a food cup, and then withdrew from the tyrosin, just as in the previous experiments. A second attempt was made to move over the tyrosin—92, 93—but the pseudopod was retracted before the tyrosin was entirely covered. Then the vestige of the previous main pseudopod became active again and the ameba moved off. The food cup that was formed was completed. It remained undiminished in size for at least thirty minutes.

A grain of tyrosin was then laid to the right of the path of another granular ameba—98. The ameba sent out a pseudopod toward the tyrosin. After it came into contact with the tyrosin the ameba formed a food cup and ingested it. At the time of closing of the food cup the ameba loosened its hold on the sub-

stratum and rolled over, contracting antero-posteriorly at the same time—107. The effect of the tyrosin on this ameba was similar to that on the previous ameba, except that the tyrosin did not seem to act so intensely or so quickly on the latter ameba. A small grain of tyrosin—T_2, Fig. 109—was then laid on the ameba but no change of behavior was observed. Another grain of tyrosin was then laid in contact with the ameba at the anterior end—112. The ameba sent out a pseudopod at the anterior end, upward into the water, and out of contact with the tyrosin; but the weight of it became so great that the ameba keeled over and so was removed from contact with the tyrosin. Another grain of tyrosin was then placed in the path of the ameba—113. The ameba moved on over it with very slight change of behavior. Another grain of tyrosin was placed in the ameba's path—117—but after slight movement toward it the original direction of movement was resumed and the ameba moved on without further change of behavior toward the tyrosin. The tyrosin grain which this ameba ate remained in it for over two hours without apparent reduction in size.

Summary of Reactions toward Tyrosin.—The behavior toward tyrosin is anomalous; no other substance, so far as known, induces similar behavior. Although the ameba seems to be strongly attracted by the tyrosin, yet when the food cup is about to close up the ameba withdraws. The negative reaction is due apparently to too intense stimulation. In the case of one ameba the impulse to withdraw did not make itself felt until the tyrosin grain was eaten. The formation of the food cup also is peculiar. Only when stimulated with tyrosin is the food cup formed by a hollowing out of the under side of the pseudopod. No other substance has been observed to produce such behavior. In at least two instances the food cup was completed although the tyrosin grain was not in it. This indicates that the formation of food cups is somewhat of the nature of a reflex. The closure of the food cups in the circumstances described above could not be explained adequately by assuming that it was due to the direct effect of the tyrosin in solution upon the surface tension or other physical property of the ameba, as Rhumbler ('98, '10) contends. Tyrosin dissolves in the body of the ameba much more slowly

than in water. A grain of tyrosin that dissolves in water in ten minutes remains in an ameba for over two hours without appreciable diminution in size. This would seem to indicate that it is not sufficient for tyrosin to go into solution in order to be assimilated, but that it must be further broken down by digestive action; unless indeed the assimilation of dissolved tyrosin goes on very slowly. It is impossible to say at present why tyrosin remains undissolved for so long in the ameba's body. Negative behavior toward tyrosin is similar to that toward other substances. In one case negative behavior was changed to positive behavior by presenting the ameba with a grain of globulin. Before the globulin was ingested the tyrosin was avoided; after ingestion, a grain of tyrosin was eaten. This is a very good illustration of the possibility of habit formation in ameba. Tyrosin can be sensed at a distance of at least 125 microns. Although it is quite likely that the ameba reacts to tyrosin in solution in the experiments described above, yet the ameba invariably moves with great accuracy toward the center of diffusion, the tyrosin grain itself. The mere presence of molecules (or ions) in solution would therefore not explain the whole behavior. Some other factor must operate such as differences in the concentration of the molecules of tyrosin in solution, as would occur in the process of going into solution, for without some such additional factor the ameba would be unable to find the solid tyrosin.

Gelatin.—Knox's Sparkling (not acidulated) commercial gelatin was employed. Only a few tests were made, owing to the experimental difficulty of handling it. One experiment only is recorded in this paper.

A small piece of gelatin was placed to the right of the path of a granular ameba—1. A small pseudopod which was extended toward it came very nearly into contact with it, when the ameba turned to the right, avoiding the gelatin—4. A small pseudopod was then thrown out on the convex side of this pseudopod toward the gelatin, but it was retracted before it came quite into contact with the gelatin. The ameba finally moved on through the vestige of the previous main pseudopod.

Arrowroot Starch Paste.—This was made by boiling starch paste with water and allowing it to cool until a rather stiff gel

was formed. A small mass of this gel was placed in the path of an ameba—303. The ameba moved forward with a little uncertainty—304, 305. Presently, however, the ameba began to move in a concerted manner and when the main pseudopod was about one fourth past the starch paste, a pseudopod was sent out toward it, but the pseudopod was retracted before it came quite into contact with the paste. The forward movement of the ameba did not seem to be disturbed by the projection of the small side pod. The behavior of the ameba toward arrow-root starch paste is very similar to that toward gelatin.

Reactions to Substances in Solution.

Solutions of certain substances were allowed to run into very fine capillary glass tubes, after which one end of them was sealed hermetically with heat. The tubes were made a centimeter or more in length so that the substance at the open end of the tube would not be affected by the heat employed in sealing the other end. The external diameter of the tubes was about twenty-five microns, and the bore about fifteen microns, but there was some variation in the dimensions of the different tubes.

The solutions were placed in small tubes, for it is necessary to localize as definitely as possible the diffusion currents from the open end of the tubes in order to be certain of the meaning of the behavior which might be observed. But even with the employment of capillary tubes the results are more or less uncertain, unless the reactions are decided or repeated often; for, the solutions being in most cases colorless, the extent of their presence outside of the tubes can only be inferred.

Peptone.—From meat. Commercial, from Eimer and Amend. As is well known, this substance is very soluble in water and has a very strong agreeable smell and "taste." The taste is salty. The action of peptone resembles that of meat extracts upon the human senses.

A capillary tube filled with a dilute solution of peptone was placed in the path of a raptorial ameba—333. The ameba moved toward the open end of the tube and formed a large food cup before coming into contact with it, but the food cup finally closed in over the open end of the tube—334. The ameba

remained quiet for about six minutes, during which time the water and the peptone solution disappeared from the food cup. The ameba then started to move away. As the posterior end passed the tube opening another food cup was started over the tube but was not completed, and the ameba then soon moved away—335. But when the tube was again laid in the ameba's path—336—another food cup was formed over it. (A control tube containing culture fluid was then placed in the path of the ameba but only slight positive behavior resulted.) The peptone tube was then shifted—337. The ameba formed a complete food cup at a distance of about 100 microns from the end of the tube—337, 338—showing conclusively that substances in solution are capable of causing the formation of a normal food cup. The ameba then started to move away to the right—339—but presently changed its course, returned to the tube—340—and enclosed it again in a food cup—341. After remaining in this position for a minute and a half, the ameba moved off. (The control tube was then again laid before the ameba but no definite change in behavior was observed.)

A fresh tube containing dilute peptone solution was placed in the path of another raptorial ameba—342. The general result was negative behavior, due possibly to the commotion produced by placing the tube in position.

Another ameba in the same dish happened to come within range of the tube—346. A pseudopod was thrown out which moved directly toward the tube opening—347—but when it came within about 100 microns of the tube it turned to the right and moved on. The small pseudopod extended toward the tube from the convex side of the ameba as it turned to the right —349—indicates a slight tendency to positive reaction. The tube was then shifted—351. The ameba threw out a pseudopod which moved toward the peptone tube for some distance, then through a new pseudopod the ameba moved off to the right—352. While passing by the tube a side pseudopod was thrown out toward it—354—but it was soon partially retracted. Very soon it was again extended—356—and after it came within twenty microns of the opening of the tube, it was retracted, and the ameba moved directly away from the tube through a new

pseudopod. The final effect of the peptone in this case was to produce negative behavior, nevertheless the attractive qualities were quite strong.

In the path of another granular ameba was placed a new tube containing a dilute solution of peptone—358. A number of very small flagellates gathered near the open end of the tube. The ameba moved forward toward the tube until it came within thirty microns of it when a pseudopod was thrown out on the right—360—which appeared destined to become the main pseudopod, but the tendency toward positive reaction gained the upper hand again and a food cup was formed over the open end of the tube together with the flagellates, by a reactivation of the previous main pseudopod—362. Ten minutes after the formation of the food cup the ameba began to make efforts to move away. The protoplasmic current was reversed several times, but finally the ameba moved on, after having been in contact with the tube for eighteen minutes. It is impossible to tell whether the flagellates had any influence on the reactions in this experiment or not.

In the majority of cases peptone in dilute solution diffusing from a small capillary tube attracts amebas. In one ameba four food cups were induced, one being completely formed at 100 microns from the open end of the tube. There can be no doubt, then, that a solution of a chemical, such as peptone, even when entirely free from the presence of a solid, is an adequate stimulus to set off the feeding reaction. It is, nevertheless, interesting to note that in the other three cases of food cup formation the solid source, that is, the tube opening, was sought and enclosed as if the chemical in solution acted only as a guide to the solid object from which the chemical was diffusing.

Egg Albumin.—Capillary tubes were filled with a solution made from Eimer and Amend's crystallized egg albumin and filtered culture fluid. This solution diffuses much more slowly than peptone. The solutions were very dilute: one part of albumin to 200 parts water.

A tube of egg albumin was placed in the path of a granular ameba—369. The ameba moved forward into contact with the tube, though apparently without being attracted toward it.

After being in contact with the tube for a few minutes the ameba moved away from it about 100 microns, but soon returned again. The whole behavior is puzzling, and may have had no relation to the diffusing albumin.

A new tube of egg albumin was placed to the right of the path of a raptorial ameba—405. As the ameba passed the opening of the tube a pseudopod was thrown out on the side directly toward the tube—407—but it was retracted when about twenty microns from the tube—408. The ameba moved on without further reaction. The tube was then shifted—410—but it was definitely avoided by the ameba. When shifted again—415—it was again avoided. When shifted the third time—422—the behavior was indifferent.

Another tube of albumin solution was placed to the right of a raptorial ameba—426. A pseudopod which was thrown out on the right moved directly into contact with the open end of the tube—432. From this pseudopod another was sent out which moved along the side of the tube, and through it the ameba moved off—433, 434. There was no attempt made to form a food cup at any time.

In the path of another raptorial ameba was placed a fresh tube of albumin—436. This ameba was very strongly attracted by the albumin, for five food cups (451, 452, 455, 466, 469) were formed over the tube opening during the forty-one minutes the ameba remained in contact with the open end of the tube. Only the first food cup was completely closed; the others were only partially formed. This is the only certain case where a food cup was formed over the albumin because of stimulation proceeding from the albumin.

To summarize: The majority of amebas experimented upon reacted with indifference, or negatively, to the egg albumin in capillary tubes. Only one reacted decidedly positively, but this one formed five food cups in succession over the open end of the tube. There can be no doubt then of the efficiency of albumin as a stimulator for setting off the feeding process; nevertheless the stimulus seems to be weak as compared with that from peptone.

Tyrosin.—A tube filled with weak tyrosin solution was placed in the path of a raptorial ameba—398. A pair of side pseudopods

were formed near the tip of the main pseudopod, the right one of which moved toward the tyrosin tube; but this pseudopod was soon arrested and retracted while the main one enlarged rapidly for a short time when it also was retracted. The pseudopod on the left then became the main one through which the ameba moved away after ingesting a flagellate—402–404. The tube was then shifted, but the resulting behavior was indifferent.

Carmine.—A capillary tube filled with a solution (not a suspension) of carmine was placed in the path of a raptorial ameba —383. The ameba moved toward the tube a short distance—384—then reversed streaming and moved away. A new tube of carmine solution was placed before another raptorial ameba —386. The ameba was disturbed in its movements but finally sent out a pseudopod toward the carmine tube—388. But when it came within about 100 microns of the tube it broke up into several pseudopods—389—and finally the ameba moved away to the left. The tube was then shifted—393. The ameba moved toward the tube a short distance, then stopped and sent out a pseudopod on either side—395. The one on the left enlarged first, but when it came near the tube it was retracted and the one on the right enlarged and carried the ameba away—396. The tube was then laid before the advancing pseudopod—397. This pseudopod was at once arrested and the pseudopod just previously retracted became active and carried the ameba away.

These experiments indicate that solid particles of carmine are very much stronger in their stimulating power than solutions of carmine. Carmine grains nearly always produce positive behavior which results in contact, while solutions induce only negative reactions. It is at present impossible to tell why there should be such a difference, for the chemical nature of the substance is presumably the same in both cases. Better technic doubtless would do much to solve this difficulty.

A number of experiments were also performed with sodium chloride in small tubes, but the results were almost exactly like those in which culture fluid was used for filling the tubes. Here also better technic would probably give interesting results.

Summary.

1. Ameba senses small particles of insoluble substances such as carbon, glass, silicic acid, etc., at a distance of from 60 to 100 microns. The reaction is nearly always positive and it consists in the turning of the main pseudopod toward the test object or in the projection of a pseudopod on the side of the main one toward the object. The side pseudopod may or may not become the main pseudopod. After the test object is touched the ameba usually moves on without further change in behavior.

2. Although the facts are clear, the explanation of reaction at a distance to insoluble substances is lacking. Surface action of some sort or the reflection of light from the test object are possible factors. But whatever the explanation, the ability of an eyeless animal like ameba to sense insoluble objects at a distance is without parallel among organisms and it is consequently a phenomenon of fundamental importance in sense physiology.

3. Ameba reacts positively to tyrosin. The behavior toward small grains of tyrosin is very peculiar. The ameba moves toward the tyrosin as if strongly attracted and begins the formation of a food cup. The stimuli then seem to become so intense that they produce a negative effect in the reactions, and the ameba withdraws from the tyrosin grain. Tyrosin grains are however occasionally eaten.

4. The reactions toward tyrosin refute completely the contention (if it still needs refutation) that the eating process in ameba is purely a surface action effect of the stimulating object.

5. Both egg albumin and peptone in weak solution diffusing from a capillary tube cause the formation of food cups. In nearly all cases the food cups were formed over the open end of the tube as the solid source of the diffusing albumin or peptone; but in one or two cases a food cup was formed at a distance from the tube. Peptone stimulates the ameba much more readily than albumin.

6. Solutions of carmine and tyrosin in capillary tubes do not readily attract amebas. This is in strong contrast to the behavior toward grains of these substances, which is almost always positive. The difference in behavior as recorded is perhaps due to faulty technic in handling the capillary tubes.

7. The experiments indicate that ameba is sensible to at least two kinds of stimulation: that from insoluble solids such as carbon and glass, and that from substances in solution, such as peptone or albumin. It seems hardly possible that carbon and glass, at a distance from the ameba, produce the same disturbance on the surface of the ameba, such as a change in surface tension, as contact with dissolved peptone. A definite conclusion however awaits further work.

BIBLIOGRAPHY.

Rhumbler, L.

'98 Physikalische Analyse von Lebenserscheinungen der Zelle. Arch. f. Entw.-Mech., Bd. 7, pp. 103–350. 100 figures. 2 plates.

'10 Die verschiedenartigen Nahrungsaufnahmen bei Amöben als Folge verschiedener Colloidalzustände ihrer Oberflächen. Arch. f. Entw.-Mech., Bd. 30, erster Teil, pp. 194–223. 9 figures.

Schaeffer, A. A.

'12 Selection of Food among Lower Animals. Trans. Tenn. Acad. Science, Vol. 1, pp. 35–43. 2 figures.

'12 Contributions to the Feeding Habits of Amœba. Trans. Tenn. Acad. Science, Vol. 1, pp. 59–64. 3 figures.

'14 Feeding Habits of Ameba. Science, Vol. 39, p. 472.

'14 Reactions of Ameba to Light. Science, Vol. 39, p. 474.

'16 On the Feeding Habits of Ameba. Journ. Exp. Zool., Vol. 20, pp. 529–584. 6 plates.

'17 On the Reactions of Ameba to Isolated and Compound Proteins. In Press. Journ. Exp. Zool.

EXPLANATION OF PLATES.

The figures are camera lucida drawings taken from the laboratory notes without alterations. The camera lucida was attached to the right hand tube of a long arm Zeiss binocular microscope. Eyepiece 4 and objective a_3 were used, giving a magnification of 65 diameters. A scale by means of which the size of the amebas and of test objects can be estimated is shown on Plate 7.

The figures are numbered serially from 1 on for reference. An x following a number, as 9x, indicates the end of the experiment illustrated by Figs. 6 to 9x inclusive. A new experiment starts with Fig. 10 and ends with Fig. 12x, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 6 to 27xx represent the results of several experiments upon the same ameba. With Fig. 28 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed upon any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In many cases the time of drawing of each figure is also given, and where it is not given it may easily be computed.

The arrows show the direction of active protoplasmic streaming. The arrows in the last figure of each experiment denote the direction the ameba took in moving away from the test object.

The test objects are labelled in abbreviated form. See table of abbreviations below. For quick and correct reference the test objects are connected with the proper ameba by leader lines. These lines have no other significance.

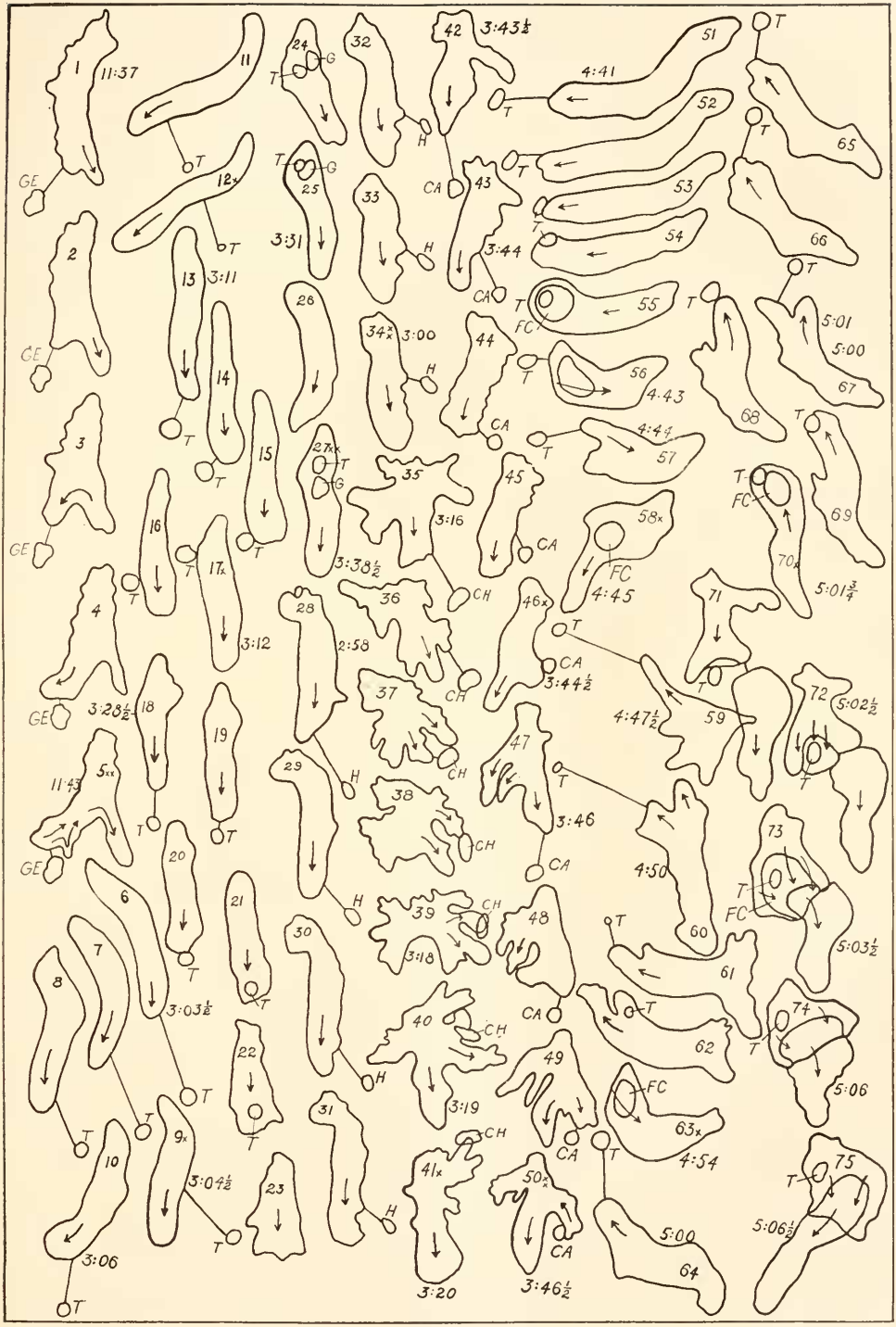
All the work was done facing a north window. All the figures were drawn in the same position in the laboratory and on the plates. The top of each plate therefore points toward the north. This is worth noting from the point of view of the possible influence of light on the behavior of ameba.

It will be noted that there are slight differences in the size and shape of the same test object as drawn in the figures of any single experiment, even if the object was not rolled around by the ameba. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see text.

Table of Abbreviations.

AR, arrowroot starch grains.
ARP, arrowroot starch paste.
C, carmine solution.
CA, carbon.
CH, cholesterin.
E, egg albumin solution.
FC, food cup.
FL, flagellates.
G, globulin.
GE, gelatin.
GL, glass.
GR, graphite.
H, hematin.
IN, indigotin.
P, peptone.
PB, lead oxide.
S, silicic acid.
T, tyrosin.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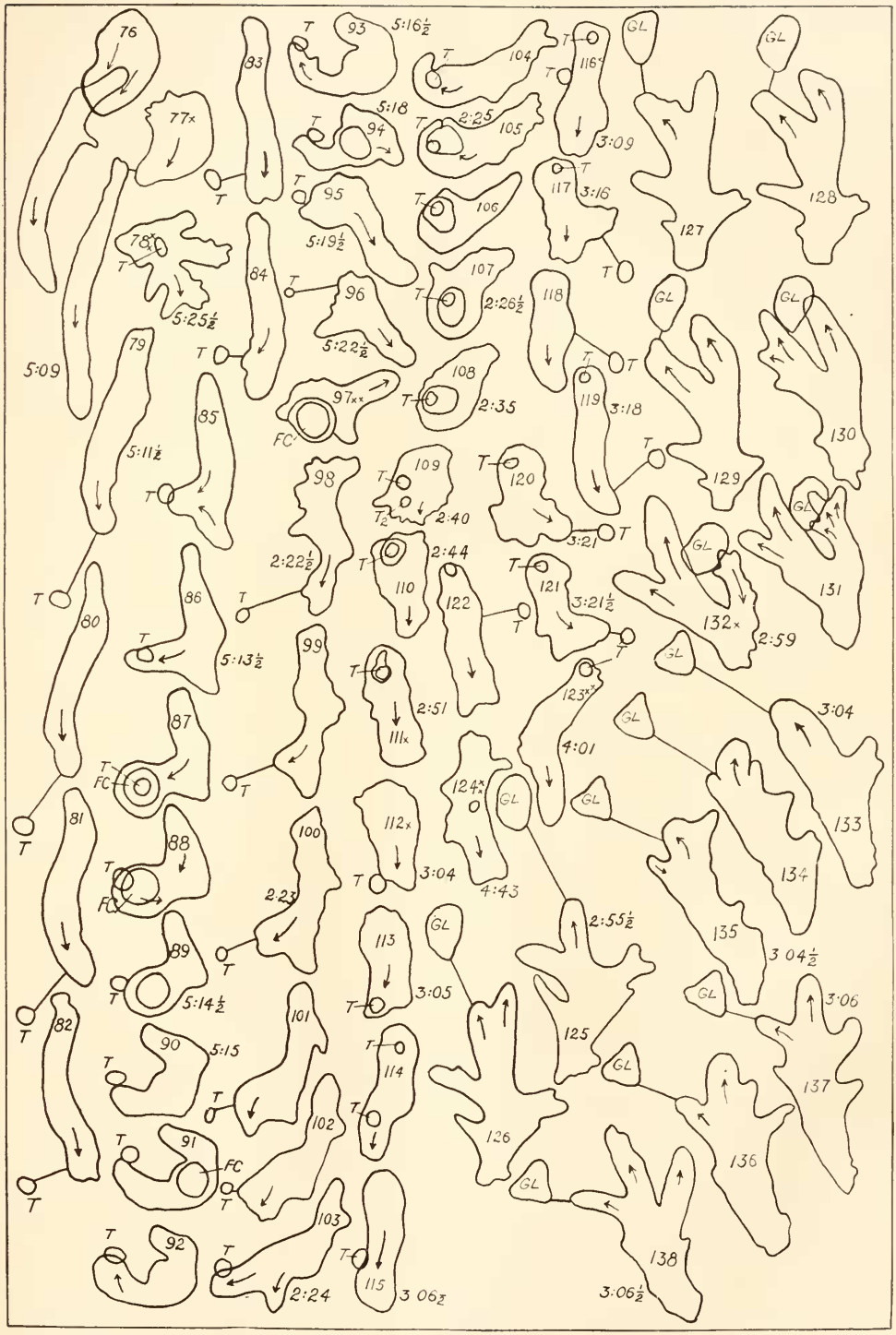

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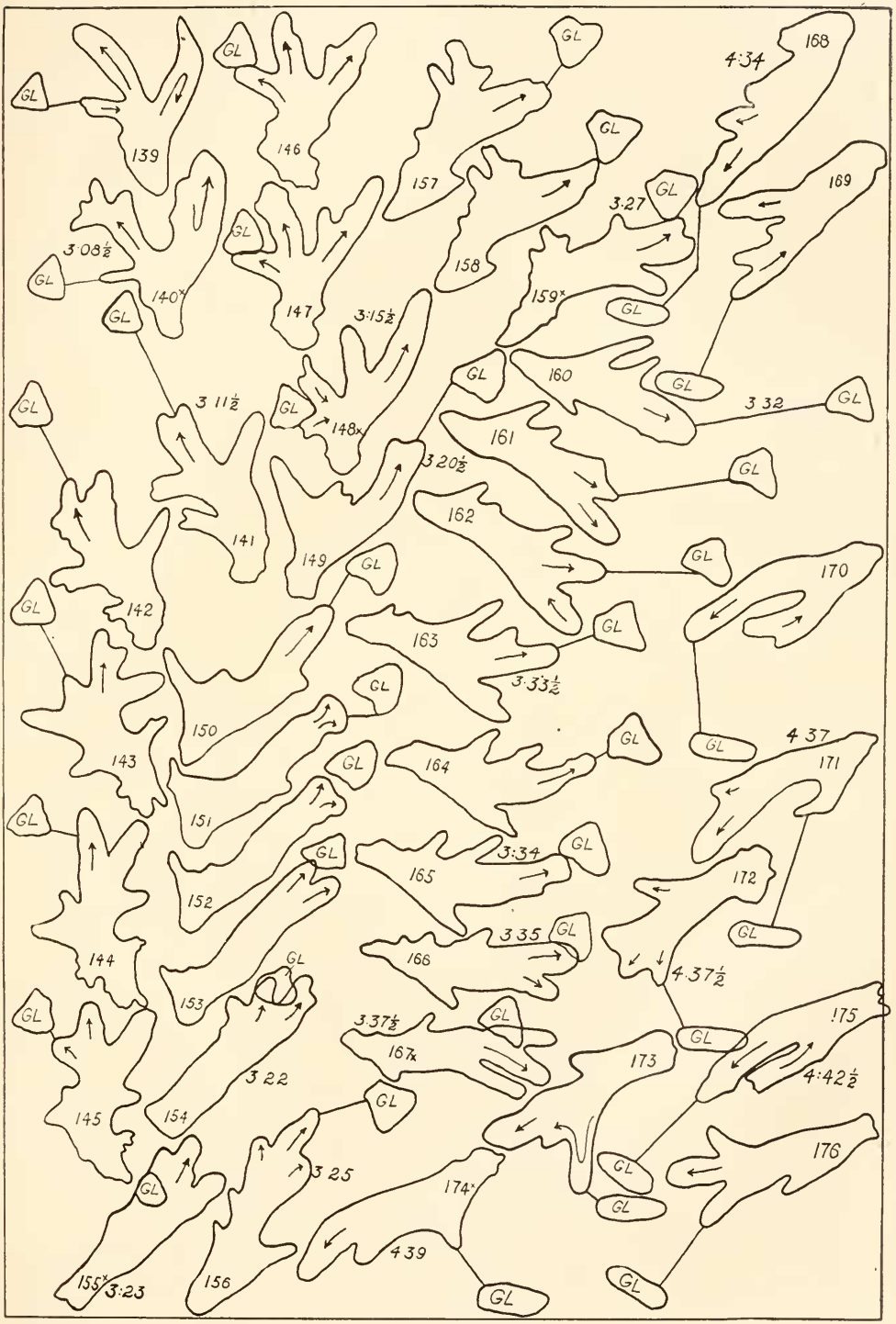

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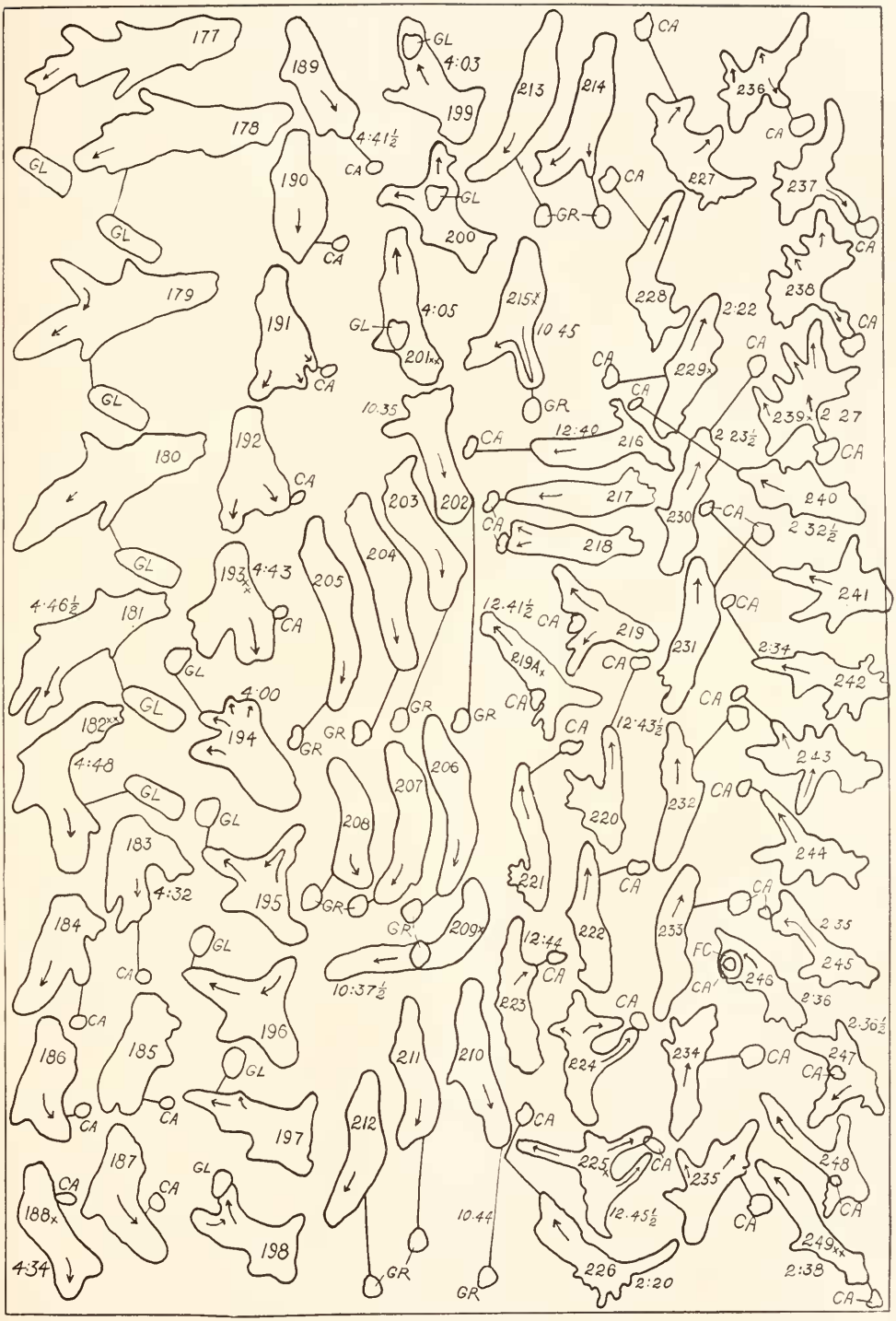

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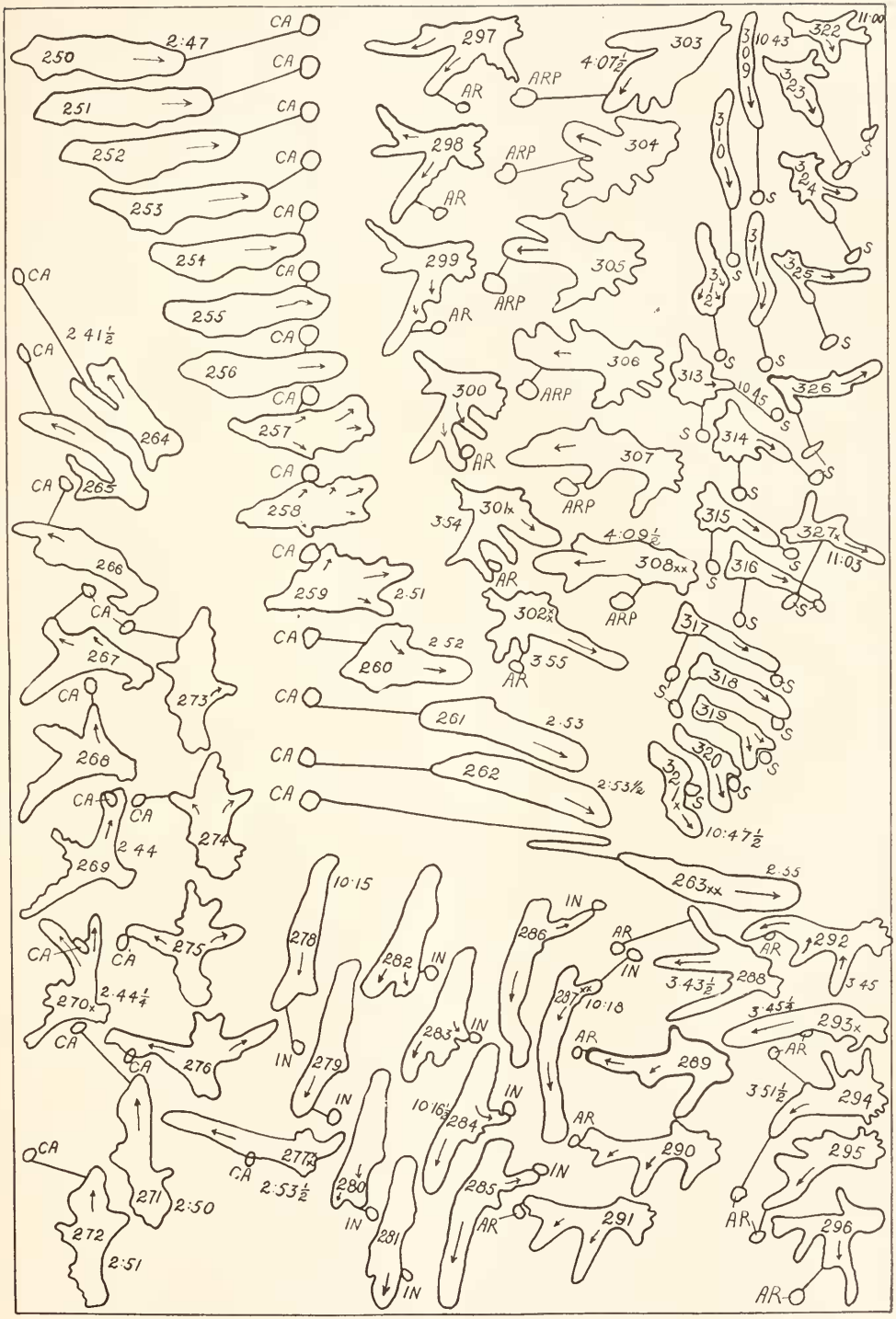

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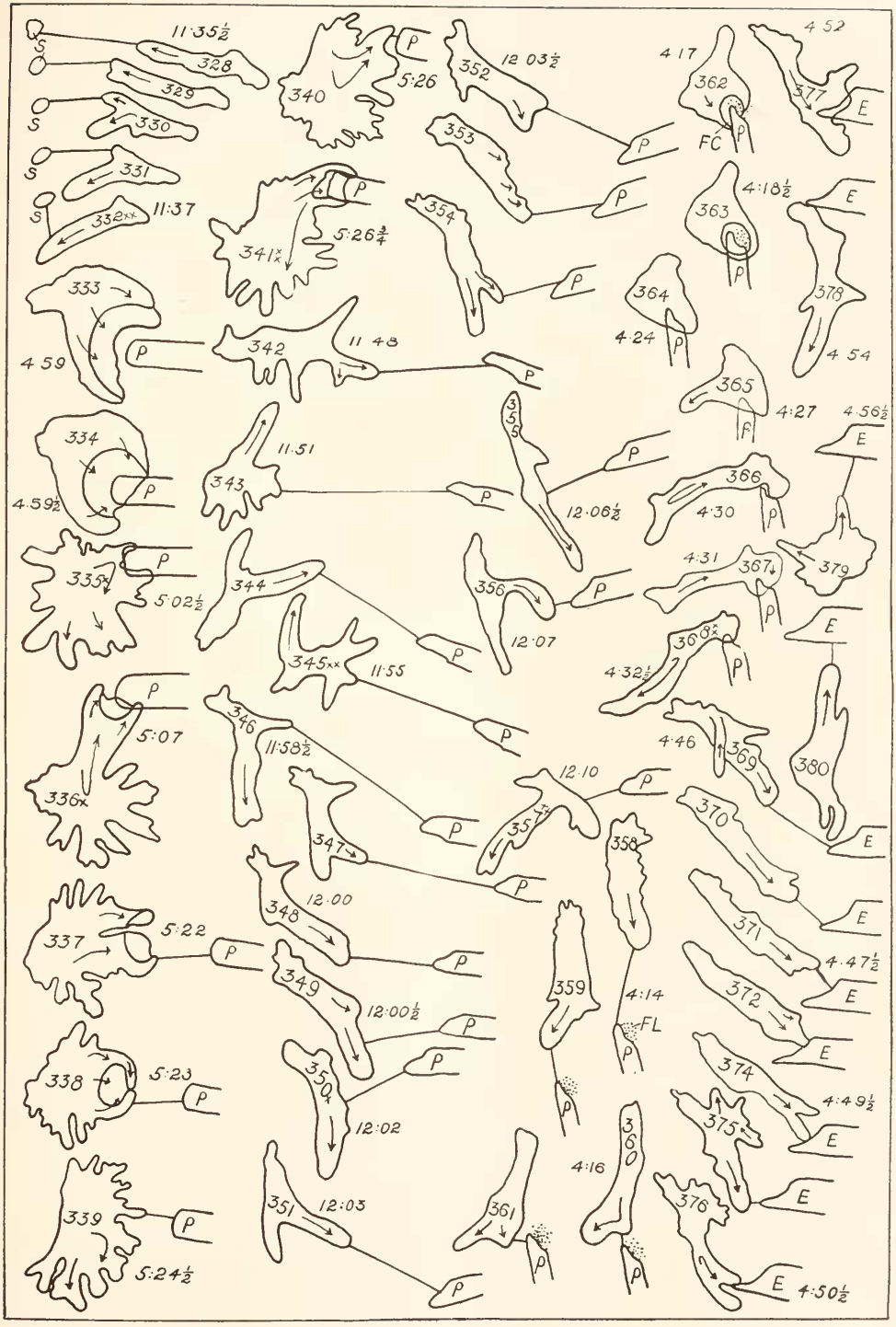

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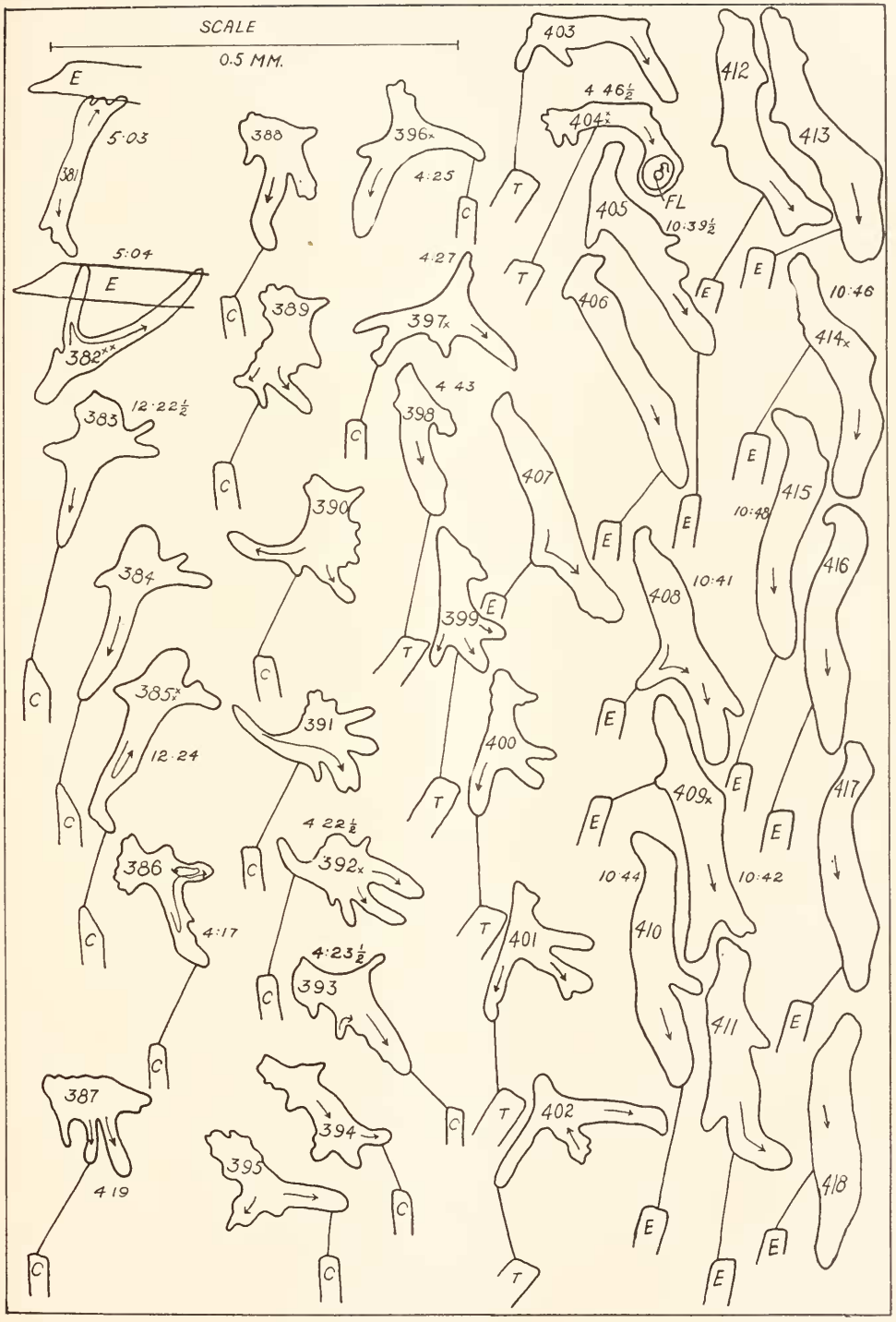

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A STUDY OF SOMATIC CHROMOSOMES.

I. The Somatic Chromosomes in Comparison with the Chromosomes in the Germ Cells of *Anasa tristis*.

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With the exception of incidental and scattered observations in connection with studies on spermatogenesis there have been only a few papers of a statistical nature on the number, type and combination of the chromosomes in somatic tissues. Usually, and more or less arbitrarily, the diploid or gonial number of chromosomes of the germ cells has been assigned to the cells of the soma. However, the number of chromosomes in the somatic cells is not necessarily the same as that in the germ cells. Cases have been reported, for instance, in *Ascaris*, some of the Hymenoptera, a Lepidopteran, and in several of the Vertebrata, where the somatic number of chromosomes is higher than the gonial number. On the other hand in *Culex*, *Diaptomus*, *Phascolosoma*, and some of the Diptera the number of chromosomes may be lower in some or in all of the cells of the body tissues than in the gonia.

Extensive studies on the development of the germ cells in animals have, in general, offered strong evidence for an "individuality" of the chromosomes. Variations in the number of chromosomes in the germ cells are relatively infrequent. This undoubtedly is of the greatest importance in the modern "chromosome theory" of heredity. At first hand the peculiar history of the chromosomes in somatic cells of certain animals may seem to render somewhat untenable this theory of the "individuality" of the chromosomes, and may easily give rise to the idea that they are exceedingly variable structures. With this in mind, a study is being made of the chromosomes in the somatic tissues of various developing embryos for the purpose of determining if chromosomes of the somatic cells generally agree in number and

type with those of the germ cells. Apparently any peculiar history of the chromosomes in somatic cells must be regarded as a concomitant of differentiation. This atypical "behavior" of the chromosomes, in lieu of a better explanation, has been satisfactorily accounted for from the standpoint of the doctrine of the "individuality" of the chromosomes in the following manner: in those forms where the chromosomes exist in the somatic cells in greater number than in the germ cells, investigators generally agree that the chromosomes of the sex cells are bivalent or plurivalent. These chromosomes fragment probably quite early in the development of the embryo, as has been shown in the case of *Ascaris* and that of the Hymenoptera. Conversely, where the number of the somatic chromosomes is lower than the number of the chromosomes in the sex cells, there is a more or less permanent pairing or fusion of the chromosomes in the soma.

Accordingly the behavior of the chromosomes in the soma can be tentatively summarized in the following manner:

1. All of the chromosomes in the gametes are univalent, and are of the same number and type in all the cells of the body, both somatic and germinal.

2. The chromosomes of either one or both of the gametes are compound, and in one of the early cleavages of the egg fragment into their component parts in the cells giving rise to the soma. This procedure clearly distinguishes the *Keimbahn* from the soma by establishing a dissimilarity between the two in the number and type of their chromosomes.

3. The production of double or multiple chromosome groups by the suppression of cell division.

4. All of the chromosomes of both gametes are univalent, but become bivalent in the cells of the soma by a more or less permanent fusion with other chromosomes. The resulting chromosomes appear univalent.

A consideration of this problem will tend to strengthen the view that the chromosomes, to a certain degree at least, are constant and not variable structures, and that they possess a certain morphological identity in the majority of animals. In the case of *Anasa*, with which this paper deals, there are certain

definite size relations among the chromosomes in the germ cells, and these size relations can be followed out successfully in the various tissues of the body.

This series of investigations was taken up at the suggestion of Prof. E. G. Conklin, and I wish to express my gratitude to him for his interest in and helpful criticism of these investigations. I am also indebted to him for the use of his slides on the spermatogenesis of *Anasa*.

Material and Methods.

The eggs of *Anasa tristis* in various stages of development were obtained in abundance on the leaves of squash plants during the months of July and August. The method of fixation, which gave the best results, was that of Carnoy and Lebrun. This fluid consists of equal parts of absolute alcohol, chloroform, and glacial acetic acid, with the addition of mercuric chloride to saturation. Eggs were well fixed in this fluid in from fifteen to twenty minutes. From this fluid the eggs were transferred to iodized 95 per cent. alcohol for twelve to eighteen hours, and were then preserved in 80 per cent. alcohol. The eggs are surrounded by an extremely tough chorion which must be removed before sectioning.

Larval ovaries were fixed for several hours in Flemming's strong solution. This same solution was also used in making preparations of the testes.

Sections of both the sex glands and the eggs were cut 7–10 micra in thickness. Owing to the presence of the yolk in the eggs, which becomes somewhat brittle after fixation, it is often of advantage to separate the embryo from the yolk before embedding, though this tendency of the yolk to crumble may be obviated to a certain degree by the addition of a small amount of crude rubber to the stock solution of paraffin.

The stain employed in all cases was Heidenhain's iron-hæmatoxylin, with or without a counter-stain of erythrosin. The value of a counter-stain is extremely doubtful, and perhaps the best preparations were obtained by a long immersion in the hæmatoxylin, which stains the chromosomes black but leaves the cytoplasm a light gray or almost colorless after destaining.

All the figures were drawn at table level with the aid of an Abbé camera lucida, using a Leitz 18 compensating ocular and a 2 mm. apochromatic objective, and are reproduced here at a reduction of one third. In a few cases it was necessary for the sake of clearness in the reproductions to draw one or two chromosomes out of their true positions. The chromosomes linearly arranged were drawn from the actual specimens, and not traced from the drawings of the plates.

Mitotic figures occur throughout the embryos and are numerous, but it must not be supposed that the chromosomes in many of these figures can be counted. It is sometimes necessary to section twenty-five or more embryos before finding a division figure which is clear. In this connection the remarks of Metz ('16, p. 215) are pertinent: "Although certain principles must be observed in making preparations, the task is mainly one of securing and preparing enough specimens to get material in the proper stages and in sufficient quantity for study."

Observations.

Anasa tristis is an exceedingly favorable form for this particular problem, since the chromosome complex is so well known, and since it has been described by several investigators with general unanimity in result. The striking size differences of the chromosomes of one plate also makes it an excellent form for study.

In order to understand the nature and character of the chromosome complex, it will be necessary to review briefly the description of the spermatogenesis and oögenesis of this species. Wilson ('05*b*) ('06) showed that the number of chromosomes appearing in the spermatogonial divisions was 21. Three of these chromosomes are larger, and two are very much smaller, than the others, so that there is a marked differentiation in size in any one group. Of these 21 chromosomes, 20 can be paired, leaving one the largest, unpaired. This largest chromosome is the *x*-chromosome (accessory). The two smallest have been termed the *m*-chromosomes, and are distinguished by the fact that they do not unite to form a bivalent chromosome in the growth period, but condense as two separate chromosomes, pairing in the first

maturation division, and immediately separating without fusion. The x-chromosome divides equationally in the first maturation, but in the second division passes undivided to one of the poles of the spindle. In this way a dimorphism of the spermatids is established, and later, a dimorphism of the spermatozoa, with respect to their chromatin content, one half containing 10, and the other half 11 chromosomes.

The oögonia on the other hand contain 22 chromosomes, four larger than the others, and two very small m-chromosomes. In the metaphase of the first oöcyte division (Morrill, '10) there are eleven chromosomes, many of them appearing as tetrads. Two of the chromosomes are larger than the others, the m-chromosomes are fused and appear as a single tetrad, and all of the chromosomes divide equally. In the metaphase of the second maturation division there are 11 dyads, two larger and one (the m-chromosome) smaller than the others.

To express, then, the possible combinations of the chromosomes at fertilization, we have the following formulæ, letting x stand for the x-chromosome, M for the macrochromosomes (the large chromosomes), *meso* for the mesochromosomes[1] (those intermediate in size), and m for the m-chromosome:

1. ♂ gamete $(x + M + 8\ meso + m)$ + ♀ gamete $(x + M + 8\ meso + m)$ = ♀ embryo $(2x + 2M + 16\ meso + 2m)$.

2. ♂ gamete $(M + 8\ meso + m)$ + ♀ gamete $(x + M + 8\ meso + m)$ = ♂ embryo $(x + 2M + 16\ meso + 2m)$.

The results summarized in these two equations were determined by Wilson and Morrill, and are confirmed by my investigations.

Wilson ('06) figures a group of chromosomes from a dividing follicle cell of the ovary. There are 22 chromosomes, corresponding in their size relations to the anticipated result mentioned above. Although he gives no figures, he states that the cells in the ectoderm of the larvæ contain approximately the same number of chromosomes as do the oögonia. He also figures a plate from a cell toward the periphery of a larval ovary in which

[1] It has become necessary to find some word by which chromosomes not characterized by their size or behavior can be designated. The term "autosome" is too comprehensive. Accordingly I have used the term *mesochromosome*, the prefix being derived from the Greek adjective μέσος meaning *intermediate in size*.

there are 44 chromosomes. This includes eight macro- and four *m*-chromosomes. He concludes, therefore, that a division of the chromosomes had taken place without a subsequent division of the cell body, and further states that such a condition is not uncommon in the investing cells of the ovary, of the oviduct, and of the fat body. These cells are considered by him as either degenerating or as highly specialized.

Morrill ('10) figures "incomplete blastoderm" mitoses in the developing eggs of *Anasa*, and finds that there are two kinds of embryos with respect to their chromosome content, one containing 21, and the other 22 chromosomes. The two different chromosome groups correspond, respectively, to the groups found in the spermatogonia and the oögonia, showing the same size differences and the same number of the various types of chromosomes. He also reports one case of 23 chromosomes, but suggests that this is due to an accident in technique.

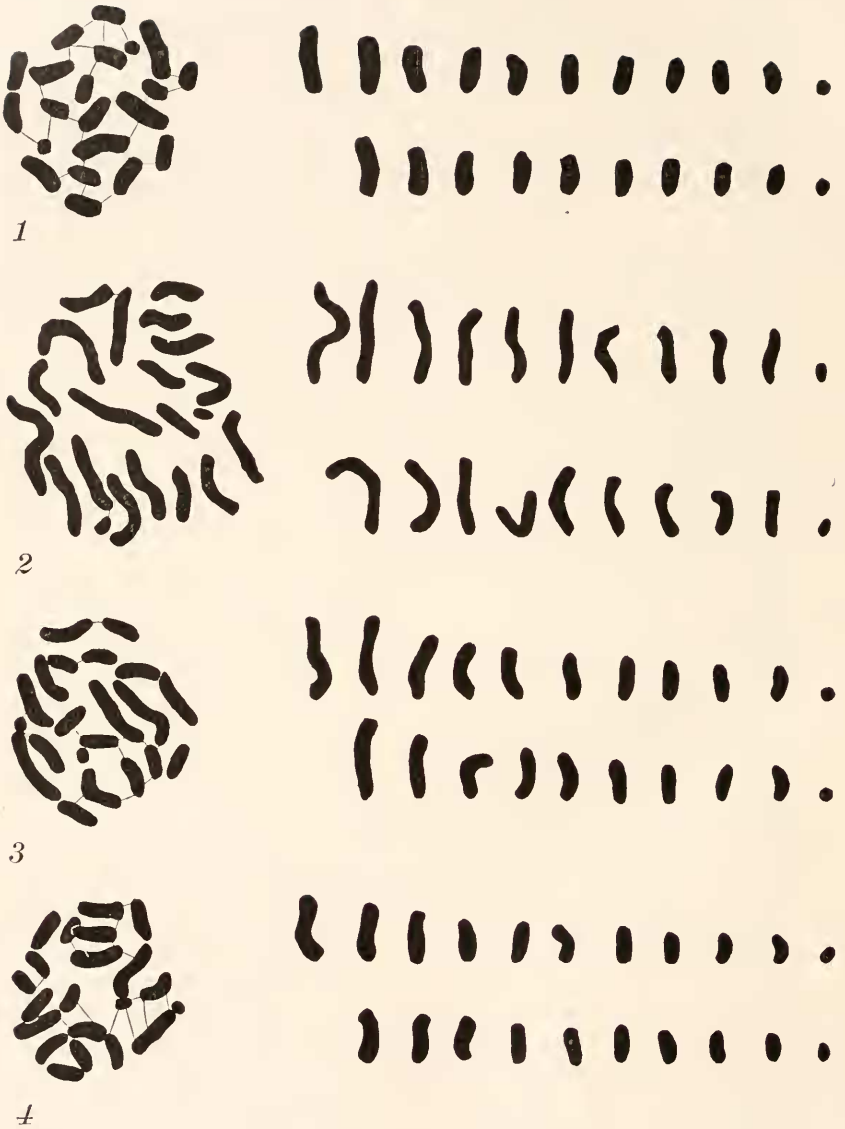
The results presented in the present paper were obtained from several stages in the developmental history of *Anasa*. These stages can be grouped, roughly, under three heads: I. Late cleavage (pre-blastodermic) and early blastodermic; II. Appearance of the limb buds, and first indications of segmentation in the embryo; III. Shortening of the elongate embryo. Of these stages, stage I. shows the clearest and most diagrammatic chromosome plates. The second stage shows division figures in the undifferentiated ectoderm, and stage III. was excellent for mitotic figures in neuroblasts, the mesoderm, and the hypodermis.

In these stages over 100 counts were made of equatorial or pre-equatorial plates. The difficulties first experienced were stated in a preliminary paper (Hoy, '14), as was also the explanation of some plates apparently containing an atypical number of chromosomes. It must be borne in mind that in some cases there appears to be a diversity in the chromosome number of different cells, but in all these plates the chromosomes are crowded together making an enumeration extremely difficult. However, there seems to be no case where an explanation of an apparently aberrant number cannot be made, logically and clearly, on the grounds of accidents in technique, overlapping of the chromosomes, or apparent fusion of several chromosomes due to poor

destaining. Foot and Strobell ('13, p. 199) have particularly objected to such explanations, characterizing them as "the old story so familiar to cytologists—if a feature is where, hypothetically, it ought not to be, it is an artifact, and if it is not where it ought to be, it is due to faulty technique." Their objections are valid, I think, up to a certain point. Variations may easily occur, in fact the double chromosome groups in *Anasa* are proof that they may occur frequently. As to the total disappearance of chromosomes I have no proof. Moreover, the metaphase plates which have been studied are all reasonably clear and no variations in the number of type of the chromosomes have been demonstrated, other than the above. It seems reasonable, therefore, not to entirely ignore the above-mentioned explanations, especially since the preponderance of proof is in favor of the characteristic chromosome number. If an entire chromosome plate lies in one section, and is sufficiently clear to be counted, the normal number has invariably been found (with the exception noted). It is well to point out that the study of prophases has been found to be unadvisable owing chiefly to the inaccuracy of the enumerations, due to the overlapping of the chromosomes.

Figs. 1–4 show plates of the 21-chromosome type. To the left are the chromosomes as they appear in the plates, and to the right the chromosomes linearly arranged. Fig. 1 is a spermatogonial metaphase. Fig. 2 a late cleavage metaphase, Fig. 3 is from a neuroblast in division, and Fig. 4 from an abdominal mesoderm cell. The last two figures are from the same embryo at a stage of development corresponding to stage II.

Bearing in mind the 21-chromosome complex previously mentioned, it will be seen that in all these figures there are 2 *m*-chromosomes and 3 chromosomes larger than the others. One of these three larger chromosomes, the largest, is obviously unpaired. In the eight pairs of mesochromosomes there is a gradual gradation in size from the macrochromosomes to the *m*-chromosomes. Aside from this decrease in size they show no striking peculiarities. The attempt to arrange the chromosomes in pairs is, of course, an arbitrary one, but it will readily be seen that, with the exception of the unpaired largest, there are two



Anasa tristis. 21 chromosomes, male type. Fig. 1. Spermatogonium. Fig. 2. Cleavage nucleus. Fig. 3. Neuroblast. Fig. 4. Mesoderm.

chromosomes of approximately the same size at each step in the gradation.

The spermatogonial chromosomes are short and thick, and the

closest resemblance to these is shown by the chromosomes of the mesoderm cell (Fig. 4). However, in both of these cases the cytoplasmic areas of the cells are much smaller than those of the cells in which the more elongate chromosomes of Figs. 2 and 3 appear. This much elongated appearance of the chromosomes is characteristic of both neuroblasts and late cleavage cells, the nuclei and cytoplasmic areas of which are larger than those of the other cells. One must also take into account the fact that the apparent size and volume of the chromosomes is influenced to some extent by the length of extraction of the stain. Realizing this, the figures produced in this paper have been taken from preparations as far as possible stained alike.

Figs. 5–8 are from embryos showing the 22-chromosome type. Here again the formula of the 22-chromosome complex, previously outlined, is confirmed. There are four macrochromosomes in each figure, the difference, as contrasted with the 21-chromosome type, being that the largest chromosome is found paired. Fig. 5 is from a late cleavage cell and, as was the case in corresponding plates of the 21-chromosome type, the chromosomes are much elongate. Fig. 6, from a neuroblast in the cerebral ganglion, was drawn from the finest preparation obtained in all the series. The chromosomes are not as large as in Fig. 3, but this figure is taken from an embryo of about stage III, while, as was pointed out, Fig. 3 is from an earlier stage.

Figs. 7 and 8 are also from embryos corresponding in their development to stage III. Fig. 8 is from a cell in the hypodermis of the antenna. This cell is of small size, and the chromosomes are the smallest of any group shown.

Fig. 9 is from an oögonial mitosis. The 22-chromosome type found in the developing embryos corresponds to this in the same manner as the 21-chromosome type corresponds to the spermatogonial plate. Fig. 10 is from a dividing cell in the oviduct, and furnishes additional proof that the 22-chromosome type is the female type, and is the number characteristic of the somatic tissues as well as the sex glands of the female.

In addition, cases of double chromosome groups were found in connective tissue cells surrounding the young ovary. These groups correspond to Wilson's report of 44 chromosomes in certain investing cells of the ovary. Since the number is double

the normal somatic number, and since the chromosome pairs are evidently doubled, it is highly probable that, as he suggests, there was no division of the cell after the division of the chromosomes. Whether these cells are degenerating or are highly specialized is of course problematical, but they do not affect the

Anasa tristis. 22 chromosomes, female type. Fig. 5. Cleavage nucleus. Fig. 6. Neuroblast. Fig. 7. Mesoderm. Fig. 8. Ectoderm.

view that the normal somatic chromosome number is a constant one in *Anasa*.

It is evident, then, that the embryos of *Anasa tristis* fall into two classes with respect to their chromosome content, one containing 21, and the other 22 chromosomes in all the cells of the various tissues where an examination was possible, with the one exception noted. These conditions are found in every plate

9

10

Anasa tristis. 22 chromosomes, female type. Fig. 9. Oögonium. Fig. 10. Epithelium of oviduct.

where it is at all possible to make an accurate count. In some cases the *m*-chromosomes lie so near the largest chromosomes that it is difficult to distinguish them. Even in metaphase groups where a count is impossible, it is possible to identify many of the chromosomes.

Aside from the general constancy in the number of the chromosomes in these embryos, the most important and significant fact is that of the constancy in type. The individuality of the chromosomes is represented by this constancy of type, and it is clear that in *Anasa* this is not confined merely to the chromosomes in the sex cells, but occurs throughout early embryonic development in the somatic tissues, and it is logical to suppose from this that this constancy in type of the chromosomes is present throughout the entire life cycle of this animal. Of similar importance is the fact that definite pairs of chromosomes can be

demonstrated in the various tissues of developing embryos. From a comparison of the diploid groups in spermatogonia and of those in oögonia, together with the haploid groups, which have been described by Wilson and Morrill, it is clearly evident that the members of these pairs of chromosomes are derived one from the male parent and one from the female parent.

Though the same size relations seen in the chromosome complex of the germ cells is reproduced in the somatic cells, there is a difference in size and volume of the homologous pairs in different cells. This difference can probably be explained upon the grounds of a greater or less metabolic activity of the nucleus; that is, difference in size may be due to a real increase in the amount of the chromatin. On the other hand it may be due merely to the swelling of the chromosomes by absorption. Conklin ('12) found that "the chromosomes of the spermatid are usually smaller than those of the oötid, but when the chromosomes of the first cleavage spindle appear, those from the sperm nucleus are usually as large as those from the egg. The reason for this is to be found in the fact that both grow, after fertilization, in the same medium, the egg plasma, and for approximately the same length of time." He found that the size of the chromosomes is dependent upon the size of the nucleus from which it comes, and that in general the small nuclei gave rise to chromosomes smaller in size than those which came from a large nucleus.

So far as has been determined, none of the chromosomes in the somatic cells are distinguished by any peculiar behavior. All the chromosomes seem to divide at about the same time, and in the same manner. The division plane is a longitudinal one, separating the chromosomes into morphological identical halves, as can be demonstrated in polar views of anaphases. In some cases the individuals of a pair lie very close together, but this is an extremely inconstant feature. Beyond a difference in size it is usually impossible to distinguish various chromosomes by any peculiarity of shape, since the same pair may appear U-shaped, slightly curved, or as straight rods in different cells, and one chromosome of a pair may even differ very much in shape from its mate. This is probably due to the position of the particular chromosome in the equatorial plate, and to some difference in tension of the spindle fibers.

Conclusions.

1. The somatic number of chromosomes in *Anasa tristis* is constant, with the one exception, namely, that double chromosome groups occur in certain investing cells of the ovary.

2. There are two classes of embryos, one with a complex of 21 chromosomes, the other with a complex of 22 chromosomes in all the cells of the various tissues of the body which have been examined, with the exception noted.

3. The chromosomes in the somatic cells are of the same number and type as those in the germ cells. The 21-chromosome type corresponds to the spermatogonial complex, and the 22-chromosome type to that of the oögonia. Accordingly, embryos having 21 chromosomes in the somatic cells are males, those with 22 chromosomes, females.

4. The male somatic chromosome complex consists of 3 macro-, 16 meso-, and 2 *m*-chromosomes, which is the same as is found in the spermatogonia.

5. The female somatic chromosome complex consists of 4 macro-, 16 meso-, and 2 *m*-chromosomes. This corresponds to the oögonial complex.

6. These facts show that there is both an identity of number and an identity of type in the chromosome complexes. The identity of type is further emphasized by the fact that definite pairs of chromosomes can be demonstrated in these groups.

7. The double chromosome groups do not represent fragmentation or transverse splitting of the chromosomes, for the female formula is doubled and this evidently has been brought about by the failure of the cells in question to divide after a division of the chromosomes had taken place.

Review of Literature.

The following table is composed of the counts which have been made of the chromosomes in somatic cells. In a great many cases these counts have been incidental to a study of the sex cells, and have served, in the main, merely to substantiate the counts of the diploid number of chromosomes in the sex cells. It is an unfortunate fact that the term "somatic number" is frequently used for that of "diploid number" of the germ cells. In a few cases it has been impossible to determine whether the

author has actually counted the chromosomes in somatic cells or has really meant the diploid number of chromosomes in the sex cells. Since the two numbers are not alike in many animals, the use of the former term in this connection should be abandoned.

This is not intended to be an exhaustive list of the literature on the subjects of spermatogenesis or oögenesis. Only where accounts do not agree is reference made to more than one author. A number of papers on oögenesis have reported counts of chromosomes in the early cleavage of the egg. For the most part these reports have not been included here, since counts of the chromosomes in the first or second cleavages can hardly be considered as conclusive of the somatic number, except where the *Keimbahn* has been determined and where definite statement is made of the particular cell in which the chromosomes were observed. When the counts made in the early cleavage are followed up by counts in the germ layers or their derivatives they then become of significance. In this connection I have omitted the counts of chromosomes in the early cleavage of Echinoderms, and of the lower worms, in both of which groups numerous counts have been made. Obviously, too, cases where only a few counts have been made in one tissue are not at all conclusive when unaccompanied by counts in other tissues. However, since these are from definite somatic tissues they have been included here.

In the alloiogenetic forms only the sexual generations are included, since the purpose of this table is the comparison of the number of the chromosomes in the sex cells with those in the somatic cells. Investigation has been made on the chromosomes of parthenogenetic individuals in a number of forms, particularly in the Hymenoptera, the Aphididæ, and in the Crustacea. (Morgan, Stevens, etc.)

Where two different numbers are given for the haploid or reduced number, this refers to the fact that an accessory chromosome (or chromosomes) is present, and where the two haploid numbers are the same, to the fact that idiochromosomes are present, thus establishing a dimorphism of the spermatozoa in both cases. Where different numbers separated by a hyphen are given under either the diploid or haploid columns, a number varying between the two has been found.

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
VERTEBRATA					
MAMMALIA					
Homo	♂ 22	♂ 10, 12[1]			Guyer ('10)
		♂ 10–12			Montgomery ('12)
		♂ 12			Duesberg ('06)
		♂ ca. 12			Jordan ('14)
		♂ 16			Moore and Arnold ('06)
			Cornea of eye	24	Flemming ('98)
			Mucus cells	32	Farmer, Moore, Walker ('05)
			Connective tissue	32	Farmer, Moore, Walker ('05)
			Lymph bodies	32	Farmer, Moore, Walker ('05)
			Liver	34	Wieman ('13)
			Brain	33, 34	"
			Mesenchyme	34, 38	"
			Intestinal mesothelium	34	"
			Nasal epithelium	34, 38	"
	♂ 47	♂ 23, 24			Winiwarter ('12)
	♀ ca. 48				"
Lepus cuniculus	♂ ca. 22	♂ 11, 11			Bachhuber ('16)
		♀ 10–12[2]			Honoré
	♂ 28–36		Epithelium of ear	28–32	Barratt ('07)
	♀ 42		Great omentum	42, 80	Winiwarter ('00)
			Amnion	42	"
			(not stated)	24	Flemming ('98)
Cavia cobaya	♂ 56	♂ 28, 28			Stevens ('11b)
			Mesoderm	etwa 24	Flemming ('98)
Canis familiaris			Leucocyte	8	vom Rath ('94)
			Epithelium of bladder	8	" "
			Epithelium of bladder	32	" "
			Epithelium of bladder	64	" "
Felis domestica	♀ 36	♀ 12	(not stated)	36	Winiwarter and Sainmont ('09)
Sus scrofa domestica	♂ 18	♂ 8, 10	♂ mesonephros	18	Wodsedalek ('13)
	♀ 20		♀ "	20	"
Didelphys virginiana	♂ 17	♂ 8, 9	Interstitial cell	17	Jordan ('11)
AMPHIBIA					
Salamandra maculosa	♂ 24	♂ 12			Meves ('97)
			Pronephros	12	vom Rath ('94)
			Yolk	12	" "

[1] Guyer reports a further reduction in the second division to 5 and 7. This is accomplished by a fusion of the autosomes.

[2] Fide Winiwarter ('00).

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Salamandra maculosa.....	♂ 24	♂ 12	Blood	12	vom Rath ('94)
			Peritoneal endothelium	19–27	Della Valle ('09)
			Epithelium	24	Rabl ('06)[1]
			Connective tissue	24	" Meves ('11)
			Epithelium of gill	24	Meves ('11)
Amblystoma.....		♀ 8	1st cleavage	16	Fick ('93)
		♀ 15	1st "	ca. 30	Jenkinson ('04)
			Cleavage	12	Kölliker ('89)[2]
			Nerve	24	Mack ('14)
			Ectoplasm	24	"
			Muscle.....	24	"
			Pigment	24	"
			Connective tissue	24	"
DIPNOI					
Lepidosiren paradoxa.....	♂ 38	♂ 19			Agar ('11)
			Larval nerve	38	" ('12)
			Mesenchyme	ca. 36	Murray ('06)
			Ectoderm	ca. 36	"
			Chorda dorsalis	ca. 36	"
			Red blood cell	ca. 36	"
MOLLUSCA					
GASTEROPODA					
Paludina vivipara.	♀ 14	♀ 7	Cleavage	14	Popoff ('07)
			Tissue cells	14	"
INSECTA					
DIPTERA					
Drosophila amœna	♂ 8		Larval, pupal and adult	8	Metz ('14)
	♀ 8				
Drosophila quinaria.....	♀ 8		Larval, pupal, and adult	8	Metz ('14)
Drosophila repleta	♂ 12		Larval, pupal, and adult	12	" ('16)
	♀ 12				" ('14)
Drosophila funebris.....	♀ 10		Larval, pupal, and adult	10	"
Drosophila tripunctata.....	♀ 8		Larval, pupal, and adult	8	"
Drosophila ampelophila.....	♀ 8		Larval, pupal, and adult	8	"
	♂ 8	♂ 4, 4	Segmentation	8	Stevens ('08*a*)
			Ovarian follicle	8	"

[1] Fide Meves ('11).

[2] Fide Jenkinson ('04).

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Eristalis tenax....	♂ 12	♂ 6, 6	Follicle of testis	12	Stevens ('08*a*)
			Ovarian follicle	12	"
Calliphora erythrocephala.....		♂ 6	Somatic	12	Metz ('16)
	♀ 12				"
Sarcophaga sarraciniæ.....	♂ 12	♂ 6			Stevens ('08*a*)
	♀ 12		Ovarian follicle	12	"
Sarcophaga sp....			" "	12	Metz ('16)
			Somatic	12	"
			"	24	"
			"	48	"
Ravinia peniculata	♀ 12		Ovarian follicle	12	"
Homalomya sp....		♂ 6	Somatic	12	Metz ('16)
Fucellia marina ..			Somatic	12	"
			"	24	"
			"	48	"
Spogostylum simson.....			Ovarian follicle	12	"
Culex pipiens....	♂ 3	♂ 3	Undifferentiated larva	3	Taylor ('14)
			Muscle	3	"
			Nerve	3	"
			♂ tracheal tube	3	"
			♂ Malphigian tube	3	"
			♀ alimentary canal	3	"
			♀ body wall	3	"
			♀ Malphigian tube	3	"
			Egg follicle	3	"
		♂ 3			Stevens ('11*a*)
	♂ 6				"
					Metz ('16)
	♀ 6				Stevens ('11*a*)
					Metz ('16)
HYMENOPTERA					
Apis mellifica....	♂ 16				Meves ('07)
		♂ 16			"
					Nachtsheim ('13)
	♀ 16	♀ 8	Cleavage	32	Nachtsheim ('13)
			Blastoderm	32	Nachtsheim ('13)
			"	64	Petrunkewitsch ('01)
Nematus ribesii ..	♂ 8	♂ 4	Developing egg	8	Doncaster ('07)
	♀ 8	♀ 4	Ovary sheath	ca. 16	"
LEPIDOPTERA					
Phragmatobia fuliginosa.....		♀ 28, 29	♀ blastoderm	58	Seiler ('14)
			♀ "	61	"

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Phragmatobia fuliginosa	♂ 56	♂ 28	♂ blastoderm	56	Seiler ('14)
			♂ "	62	"
Lymantria japonica		♂ 31	Blastoderm	62	"
		♀ 31			"
Lymantria dispar		♂ 31	Blastoderm	62	"
		♀ 31			"
Philosamia cynthia	♂ 26	♂ 13			Dederer ('07)
		♀ 13	Blastoderm	26	" ('15)
COLEOPTERA					
Tenebrio molitor	♂ 20	♂ 10, 10	♂ pupa	20	Stevens ('05)
	♀ 20		Follicle of egg	20	"
Elater "I"	♂ 19		Follicle of egg	20	" ('06)
Chelymorpha argus	♂ 22	♂ 11, 11	Follicle of egg	22	"
Odontota dorsalis	♂ 16	♂ 8, 8	Wall of testis	16	Stevens ('06)
Trirhabda virgata	♂ 28	♀ 14, 14	♂ pupa	28	"
			Egg follicle	28	"
Trirhabda canadense	♂ 30	♂ 15, 15	Egg follicle	30	"
Diabrotica vittata	♂ 21	♂ 10, 11			" ('08*b*)
		♀ 11	Ectoderm	22	Hoy ('14)
			"	21	"
			Neuroblast	21	"
Photinus pennsylvanicus	♂ 19	♂ 9, 10	♂ digestive tract	19	Stevens ('09)
	♀ 20				"
Photinus consanguineus	♂ 19	♂ 9, 10			"
	♀ 20		Egg follicle	20	"
ORTHOPTERA					
Gryllus domesticus	♂ 21				Gutherz ('07)
					Baumgartner ('04)
		♂ 10, 11			Baumgartner ('04)
			♂ somatic	20	Gutherz ('07)
			In larval ovary	20	"
Leptophyes punctatissima	♂ 31		Intestinal epithelium	31	Mohr ('15)
	♀ 32		Oviduct	32	"
Locusta viridissima	♂ 29		♂ somatic	29	Mohr ('15)
	♀ 30		Oviduct	30	"
Steiroxys trilineata	♂ 29	♂ 14, 15	Epithelium of vas deferens	29	Davis ('08)
Acridium granulatus	♂ 13	♂ 6, 7	Epithelium of mesenteron	13	Robertson ('16)
			Proctodeum	13	"
			Hypodermis	13	"
			Fat body	13	"
			Connective tissue	13	"

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Acridium granulatus	♂ 6, 7		Follicle of testis	13	Robertson ('16)
			Giant cell-fat body	26	"
			Ovarian follicle	14	"
Acridium incurvatus		♂ 6, 7	Follicle of testis	13	"
Tettigidea parvipennis	♂ 13	♂ 6, 7	♂ fat body	13	"
	♀ 14		Ovarian follicle	14	"
Pamphagus marmoratus	♂ 19	♂ 9, 10	Ovarian follicle	20	Granata ('10)
Aploplus mayeri	♂ 35	♂ 17, 18	Egg follicle	36	Jordan ('08)
Leucophæa maderiæ	♂ 23	♂ 11, 12			Morse ('09)
	♀ 24		Follicle of ovary	24	"
Periplaneta americana	♂ 32	♂ 16	Body tissues	32	Farmer and Moore ('05)
	♂ 33	♂ 16, 17			Morse ('09)
	♀ 34		Ovarian follicle	34	"
Blatta germanica	♂ 23	♂ 11, 12			Wassilief ('07)
	♀ 24		Egg follicle	24	"
Anisolabis maritima	♂ 24	♂ 12, 12			Randolph ('08)
	♀ 24		Egg follicle	24	"
HEMIPTERA					
Philænus spumaria	♂ 23	♂ 11, 12	Follicle	23	Boring ('13)
	♀ 24		♀ somatic	24	"
Aphrophora quadrangularis	♂ 23	♂ 11, 12	♂ larva	23	Stevens ('06)
Pœciloptera septentrionalis	♂ 27	♂ 13, 14	♀ somatic	28	Boring ('07)
Pœciloptera pruinosa	♂ 27	♂ 13, 14	Egg follicle	28	"
Galgulus oculatus	♂ 35	♂ 16, 19			Payne ('08)
	♀ 38		Ovarian follicle	38	"
Diplocodus exsanguis		♂ 13, 13	♂ somatic	26	Payne ('09)
			♀ somatic	26	"
Fitchia spinosula	♂ 27	♂ 13, 14	♀ somatic	28	"
Prionidus cristatus	♂ 26	♂ 12, 14	♀ somatic	28	"
Sinea diadema	♂ 28	♂ 13, 15	♀ somatic	30	Payne ('09)
Acholla multispinosa	♂ 26	♂ 11, 15			" ('10)
	♀ 30		♀ somatic	30	"
Pyrrhocoris apterus	♀ 24	♀ 12	Connective tissue	24	Henking ('92)
			Body cells	24	"
			Ovarian follicle	24	Gross ('07) Wilson ('09*c*)
	♂ 23	♂ 11, 12			Wilson ('09*a*)
Protenor belfragei	♂ 13	♂ 6, 7			" ('06)
	♀ 14				" Morrill ('10)

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Protenor belfragei.		♀ 7	Cleavage	14	Morrill ('10)
			"	14	"
			Ovarian follicle	14	Wilson ('06)
Syromastes marginatus.....	♂ 22	♂ 10, 12	Ovarian cells	24	Wilson ('09*c*)
Anasa tristis.....	♂ 21	♂ 10, 11			Wilson ('06)
	♀ 22	♀ 11			Morrill ('10) Wilson ('06)
			Cleavage	21	Morrill ('10) Hoy
			"	22	Morrill ('10) Hoy
			Ectoderm	21	Hoy Wilson ('06)
			"	22	"
			Mesoderm	21	"
			"	22	"
			Hypodermis	21	"
			"	22	"
			Neuroblast	21	Hoy
			"	22	"
			Ovarian follicle	22	Wilson ('06)
			Investing cell of ovary	44	"
Archimerus alternatus.....	♂ 15		Cleavage	15	Morrill ('10)
	♀ 16	♀ 8	"	16	"
Chelinidea vittigera			Cleavage	21	"
			"	22	"
Alydus pilosulus..	♂ 13	♂ 6, 7	Investing cell of testis	13	Wilson ('06)
	♀ 14		Egg follicle	14	"
Metapodius terminalis.....	♂ 21–26	♂ 11–16	Ovarian cells	22–25	" ('09*b*)
Metapodius femoratus.....	♂ 22–28	♂ 12–16	" "	23–28	"
Metapodius granulosus.....	♂ 22–27	♂ 12–17	" "	25–26	"
Podisus spinosus.	♂ 16	♂ 8, 8			" ('05*a*)
			♀ follicle cell		" ('06)
Euschistus crassus	♂ 12		♂ embryo	12	Foot and Strobell ('12)
	♀ 12		♀ "	12	Foot and Strobell ('12)
Cœnus delius.....	♂ 14		♀ Follicle cell	14	Wilson ('06)
ODONATA					
Anax junius.....	♂ 27	♂ 13, 14	Follicle of ovary	28	Lefevre and McGill ('08)
Prototracheata					
Peripatus balfouri	♂ 28	♂ 14	Yolk cell of testis sheath	19	Montgomery ('00)
	♀ 28		Blood cell	ca. 26	Montgomery ('00)
			Endoderm	28	Montgomery ('00)

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Peripatus balfouri			Ectoderm	28	Montgomery ('00)
			Ovarian stalk cell	34	Montgomery ('00)
Crustacea					
Branchipus grubei		♂ 12	Cleavage	24	Fries ('09)
	♀ 24	♀ 12	Intestinal epithelium	24	"
Diaptomus cœruleus	♂ 28		Intestinal epithelium	14–28	Krimmel ('10)
	♀ 28		Nerve cells	14–20	"
			Muscle	14–28	"
			Hypodermis	14–20	"
Annelida					
Phascolosoma vulgare		♂ 10	Late cleavage	10	Gerould ('06)
		♀ 10	Ectoderm of gastrula	10	"
Ophryotrocha puerilis	♂ 4		Ectoderm	4	Korschelt ('95)
	♀ 4		Mesoderm	4	"
			Endoderm	4	"
			Blastula	4–8	"
	♂ 8	♂ 4	Somatic	8	Grégoire and Deton ('06)
Nematoda					
Stongylus paradoxus	♂ 11	♂ 5, 6	Ectoderm	11	Gulick ('11)
	♀ 12	♀ 6	Endoderm	11	"
			Endoderm	12	"
Angiostomum nigrovenosum	♂ 11	♂ 5, 6	♂ 1st and 2nd cleavage	11	Schleip ('11)
	♀ 12	♀ 6	♂ blastula	22	"
			♀ 1st and 2nd cleavage	12	"
			♀ late embryonic stage	24	"
Ancyracanthus cystidicola	♂ 11	♂ 5, 6	Cleavage	11	Mulsow ('12)
	♀ 12	♀ 6	"	12	"
Ascaris megalocephala (univalens)	♂ 2	♂ 1			Brauer ('93)
	♀ 2	♀ 1	Cleavage	ca. 60	Boveri ('87) ('99)
Turbellaria					
Planaria simplissima		♂ 3–4	Somatic cell	6	Stevens ('04)
		♀ 3–6			"
Trematoda					
Zöogonus mirus		♂ 5	Cleavage	10	Goldschmidt ('05)
	♀ 10	♀ 5	Tissue cells	10	Goldschmidt ('05)

Group, Genus, Species.	Chromosomes in Sex Cells.		Somatic Cell.	No. of Chromosomes.	Authority.
	Diploid No.	Haploid No.			
Zoögonus mirus...	♀ 10	♀ 5	Epithelium of vas deferens	10	Goldschmidt ('08)
			Parenchyme	10	Goldschmidt ('08)
			Epithelium of bladder	10	Goldschmidt ('08)
	♀ 12	♀ 6	Interstitial	12	Wasserman ('13)
			Embryonic	12–14	"
	♂ 12	♂ 6	Cleavage	12	Grégoire ('09)
		♀ 6	Ectoderm	ca. 12	"
Hydrozoa					
Gonionemus murbachii.....	♂ ca. 24	♂ 12	Cleavage	24	Bigelow ('07)
	♀ ca. 24		Endoderm	24	"

The chromosome number appears to vary in a variety of forms. Many reports are apparently antagonistic. It may be that in certain animals the chromosome number differs in different individuals. Evidently much fruitful work can be done in attempting a solution of such differences.

The chromosome counts in man are difficult of explanation due to the diverse and conflicting results obtained in studies of spermatogenesis. On the face of the evidence it would almost appear that the chromosome number varies. Since Guyer and Montgomery, as well as Jordan, worked on negroes and their results approximate, and since Winiwarter obtained his results from a white man, it has been suggested (Guyer, '14, and Morgan, '14) that the number of chromosomes differs in the two races. If this is true, hybrids might show an intermediate number of chromosomes. Wieman's results, however, contradict this, unless there is a synpasis of some of the chromosomes in somatic cells. Outside of Flemming's count of 24, the counts in somatic tissues show a number of chromosomes varying between 32 and 38. Moore and Arnold report a haploid number of 16. This approaches most nearly to these numbers in the somatic cells. Jordan does not hold to the exact haploid number of 12, for though the number is not lower in his preparations, he states that it may be higher by several more chromosomes. Wilcox

('00) reports a number varying between 15 and 19, though he considers 18 as the normal number. However, he does not state if this number is the haploid or diploid; the inference is that it is the former. The counts of somatic chromosomes reported by Farmer, Moore and Walker were made from cells in a normal rectum in comparison with cancerous tissue. Wieman worked on a white human embryo. He points out the fact that the number is at variance with that reported by most investigators as the diploid number of the sex cells, and suggests that the varying counts which he made in the soma were due to a breaking up of some of the larger chromosomes. The difference between the somatic and spermatogonial numbers may be due, he thinks, to a similar process. Wieman also suggests that a doubling of the chromosomes may have taken place in Winiwarter's material. Precocious division of some of the chromosomes may also give this result.

In the somatic cells of the rabbit Winiwarter obtained a chromosome number varying beween 40 and 80. The majority of counts showed 42, and this he considers the normal number. This number corresponds to the number of chromosomes in the oögonia, but a haploid number of 10–12 in the maturation divisions of the egg was reported by Honoré, one of Winiwarter's students. This latter count closely approximates the observations of Bachhuber on the number of chromosomes in the spermatogonia. Winiwarter draws an analogy to the case of *Ascaris*. However, the analogy will only hold if the "oögonium" was in reality a follicle cell or some other non-germinal cell, otherwise it would seem to be analogous to the double reduction reported by Guyer in man and the domestic fowl. Apparently the cell in question was not an oögonium. Flemming modifies his number of 24 chromosomes by the statement that the number may be even higher. Barratt's numbers are intermediate between the two extremes. With reference to the varying number of chromosomes which he reports, he suggests that such variation may be entirely normal and occur regularly.

Flemming quotes Bardeleben's count of 16 chromosomes in the spermatogonia of the guinea pig, but fails to find a number corresponding to this in a study of an embryo. His number of

24 is only approximate. Taking Miss Stevens's results as a standard this number would seem to indicate a number of chromosomes in somatic cells corresponding to the haploid number in the germ cells.

Vom Rath's observations on the dog were made on a three weeks' old animal. He qualifies his remarks with the statement that owing to the small size of the chromosomes he was able to make only an approximate enumeration. In addition to the variations in the blood cells and the bladder he found great variation in the spleen, the bone marrow and in the testis. He considers 32 as the typical number, and points out that this number, together with the variations, is a multiple of eight, which number he also found, thus showing that the chromosomes may be bivalent, quadrivalent, or even polyvalent. The fact that he frequently found multipolar mitoses seems to indicate that his material may have been abnormal.

The observations of Winiwarter and Sainmont on the cat are comparable to those of the former on the rabbit. The probability here is that there is a breaking up of the chromosomes in the somatic cells.

Vom Rath considers the chromosomes in the somatic tissues of *Salamandra* as bivalent. However, his statement "hatte ich . . . mit absoluter Sicherheit nur 12 Schleifen (Aequator 24) gefunden" is not clear. Rabl and Meves conclude that chromosome pairs cannot be distinguished in the somatic cells. Della Valle, as a result of finding a chromosome number of between 19 and 27, considers the number variable and the chromosomes as temporary and variable organizations of chromatin, which appear in the prophase and dissolve in the telophase. The number of the chromosomes is simply the quotient of the quantity of the chromatin.

Mack states that the number of chromosomes in the somatic cells of *Amblystoma* agrees with the number in the primary spermatocytes. Jenkinson merely states that his observations are not in accord with those of Fick and Kölliker.

With reference to Murray's count of 36 chromosomes in *Lepidosiren* Agar says (p. 5): "Murray gave the somatic number as probably thirty-six, which is as near the right number as could

be expected to be arrived at from the somatic mitoses with their long chromosomes."

In the Diptera Metz has reported that the somatic chromosomes are closely paired together. In the case of *Sarcophaga* and *Fucellia* the groups of 24 and 48 chromosomes represent multiple groups, and the arrangement of the chromosomes is in tetrads rather than in pairs.

In her paper on *Culex pipiens* Miss Taylor takes up in detail the work of Miss Stevens on *Culex pungens*, and is evidently unaware of the fact that Miss Stevens published a paper on *pipiens* a few years later. Miss Taylor considers the presence of six chromosomes in Miss Stevens's material as due to a precocious splitting or division of the diploid number of three. Miss Stevens described a parasynapsis or side-by-side union of the chromosomes in each cell generation. This Miss Taylor thinks may offer a clue to the conditions she found in *pipiens*, namely that in this species "parasyndesis" is converted "into actual fusion, thus resulting in the formation of three out of six chromosomes." She offers as an alternative the suggestion that one of the pronuclei does not take part in development. Her results are not entirely convincing, especially since she found stages in many of the somatic cells comparable to synizesis stages in spermatogenesis. This seems to show that perhaps her material was not of the best. Metz has found that the method of securing the preparations of the testes employed by Miss Taylor, namely that of fixing either the whole or a large part of the insect entire, results in a "clumping or running together of the chromosomes, which is exactly the kind of behavior that would cause pairs to give the appearance of single chromosomes" (p. 226). He agrees with Miss Stevens's observations.

In the spermatogenesis of the bee, according to Meves, there is no reduction division. Nachtsheim considers 32 as the normal number. The 16 chromosomes in the oögonia are bivalent, likewise the 8 which form the haploid number. Similarly a haploid number of 8 chromosomes has been reported in the spermatoctyes, which is due to this "Chromosomenkoppelung." Here these also would be bivalent. Though the reports of several investigators tend to show that the number of chromo-

somes varies in the bee, the number is always eight or a multiple of this. This agrees with Petrunkewitsch's theory that the 64 chromosomes, which he found in cells of the blastoderm of fertilized eggs, were due to a splitting up of quadrivalent chromosomes. A similar fragmentation appears to be characteristic of parthenogenetic individuals, *i. e.*, the drones. This is also the case in other Hymenoptera, as Armbruster reported in *Osmia*, and Granata in *Xylocopa*.[1]

In *Nematus* Doncaster suggests that perhaps the chromosomes of the germ cells "may be compound and consist of a number of smaller units which become separated in somatic cells" (p. 107).

The case of *Phragmatobia* is interesting since in this form Seiler has demonstrated a dimorphism of the eggs with respect to their chromosome content. The female diploid complex is composed of an *x*, 2 *y*'s, and 54 autosomes. The counts made in the blastoderm of developing eggs can be analyzed as follows: The 58 complex is due to a splitting in two of the large *y*-chromosome, and is from a female. The complex with 61 chromosomes is also from a female in which the *x*-chromosome has split up into four segments and the large *y*-chromosome has also split up into two parts. The complex with 62 chromosomes is from a male where the two *x*-chromosomes have each split up into four segments. "This striking explanation has absolutely nothing improbable about it, since a splitting of the sex chromosomes in the somatic nuclei has been observed quite often" (trans).

In *Gryllus domesticus* Gutherz found that the accessory chromosome is absent in the cells of the soma. This can be explained, he says, only by assuming a "Diminutionsprozess" or by assuming that since the accessory chromosome does not participate in the early stages of development, it does not make its appearance until later. Either of these explanations, he thinks, places the theory of the individuality of the chromosomes in an extremely delicate position.

Robertson considers that the cells in *Acridium* containing 26 chromosomes are double cells with double sets of cell organs. These may have been produced by the fusion of two cells or by the suppression of cell division after nuclear division had taken place.

[1] Fide Buchner (Referate), Arch. f. Zellf., Bd. 5, H. 3 (1910).

Farmer and Moore do not hold strictly to the number of 32 chromosomes in the cells of the cockroach. Cells were found which differed by one or several chromosomes.

Variation in the number of chromosomes in *Metapodius* affects only one class of chromosomes, the "supernumeraries." Wilson considers the 22-chromosome type as the typical. This type possesses an unequal pair of idiochromosomes. The 21-chromosome type is derived by the disappearance of the small idiochromosome. The numbers above 22 are due to the presence of one to six supernumeraries. This variation of the chromosome number is chiefly between different individuals, for owing to the irregular distribution of these supernumeraries in the maturation divisions of the sperm cells, the number each sperm receives is variable. Further variations may occur in individual cells.

The count of 19 in *Peripatus* may be due, according to Montgomery, to the fact that not all of the chromosomes were contained in one section. This may also answer for the count of 26. The count of 34 was made from two sections, and he suggests that it is possible that parts of some chromosomes were in both sections. In this way some of the chromosomes were counted twice.

Krimmel finds that in the somatic tissues of *Diaptomus* the number of the chromosomes varies between the reduced and the diploid number. This is brought about by fusion of some or of all of the chromosomes into tetrads, or the chromosomes may appear bi-partite. "Man wird hier wie bei den generativen Zellen daran denken dürfen, dass in diesen Fällen die Einknicknung oder scheinbare Querkerbe der in der Reifungsperiode auftretenden Querkerbe entspricht, dass also das Zeichen der synthetischen Aneinanderfügung zweier Chromosomen noch nicht verschwunden wäre" (Krimmel, '10, p. 790).

Gerould says: "In *Phascolosoma vulgare* I have uniformly found 10 chromosomes in the late cleavage and in the gastrula . . . each of which I regard as bivalent" (footnote, p. 87).

In addition to the cells containing four chromosomes in *Ophryotrocha*, Korschelt found eight chromosomes in some of the cells of the blastula. He believes that this number is due to a transverse splitting of the four chromosomes. Grégoire and

Deton, however, claim that eight and not four is the normal and characteristic number.

In *Angiostomum* the chromosomes fragment in the cells of the soma. The chromosomes of the sex cells are apparently bivalent.

The chromosomes of the somatic cells in *Ascaris* are derived from a fragmentation of the compound chromosomes of the sex cells. This fragmentation is accompanied by a "chromatin diminution."

Miss Stevens concluded in the case of *Planaria* that two interbreeding forms were present. The pairing of two individuals with different chromosome numbers would explain, she writes, the varying chromosome numbers she obtained.

Wasserman and Grégoire have disputed the counts of Goldschmidt in *Zoögonus*. It may be that two different varieties have been concerned, one with a chromosome complex of 10, and the other with one of 12 or more.

In considering the foregoing cases it is apparent that in general the behavior of the chromosomes in the somatic cells can be classified in the manner stated in the introduction to this paper, namely that (1) the somatic and gonial chromosomes agree in number and type, (2) the number of the chromosomes in the somatic cells is higher than in the germ cells, due to a splitting or fragmentation, (3) multiple chromosome groups are produced by the suppression of a cell division, and (4) there is a synapsis, more or less permanent, of all or certain pairs of chromosomes in each cell generation. It is also evident that there are numerous cases which are exceedingly perplexing, and which cannot be classified at the present time.

The majority of animals, particularly the insects, in which group more work of a cytological nature has been done than perhaps in any other, can be included in the first category. But even here the classification is not sharp and distinct for many forms may show at the same time the third type of chromosome behavior in one or several tissues. This, however, does not seriously affect the main conclusion, and is possibly to be considered as no more than a normal variation.

When a sufficiently detailed study shall have been made of the somatic chromosomes where some or all of these are represented

in the germ cells by compound chromosomes, it is highly probable that the number of the chromosomes will be found to be the same in all of the somatic tissues. In this type of somatic chromosome behavior we find two distinct cases, one where, as in *Phragmatobia*, there is only a splitting up of one or at the most a few chromosomes, and the other where all of the chromosomes in the germ cells are apparently compound, and each breaks up into its component parts in the somatic cells (Hymenoptera, Nematoda, and perhaps some of the vertebrates).

The synapsis of homologous pairs of chromosomes in somatic cells was described by Miss Stevens ('08, '10) in the case of a number of species of the Diptera. This synapsis apparently took place in the anaphase or telophase and lasted until the metaphase of the next division. Metz ('14, p. 55–56) confirms this: "The Drosophilas offer some of the most striking evidences of the actual pairing of chromosomes in the diploid groups, thus far observed. The phenomenon is apparently characteristic of all Diptera, but is nowhere so striking as in this genus. . . . But the most remarkable feature of the whole study is the discovery that the chromosomes not only exhibit a close association in pairs at nearly all times, but that before every cell division the members of each pair become so intimately united that they may be said actually to conjugate. Each pair, with the possible exception of the sex-chromosomes, goes through what amounts to a synapsis in every cell generation, so that in many cases the figures closely resemble those of the haploid groups. Apparently this takes place in every prophase, but a second conjugation may occur during metaphase, just a short time before division." This latter statement he qualifies in his later paper saying that it is "of only occasional occurrence and is not a uniform stage in chromosomal activities" ('16, p. 230). In this paper he presents the results of his study of this chromosome pairing in about 80 species of Diptera, where he has found it to be of uniform occurrence throughout the developmental history of the individual. He concludes that this paired arrangement is "selective to the highest degree," that the members of the pair represent, one a maternal and one a paternal chromosome, and that this pairing shows that the chromosomes (forming a pair) are qualitatively,

i. e., physico-chemically, similar. This latter idea is further developed, and he thinks that this assorting of the chromosomes in pairs demonstrates a qualitative difference of the *pairs*.

The view that the chromosomes may be the same in number and type in all the cells of the body, or that fragmentation or transverse splitting on the one hand, and fusion of pairs on the other hand may account for differences is challenged by a number of investigators, who believe that a limited degree of variation may really occur (Barratt, Gutherz, Farmer and Moore, Della Valle, Rabl, Meves, Foot and Strobell). If there may be variation in the number of the chromosomes in various cells of the same individual, it is certainly not of general occurrence, and no really clear case has been presented. I believe that variation in number is more often between individuals of the same species than between cells or tissues of the same animal. Variations in the size of chromosomes are more difficult of analysis, and more difficult of demonstration. In *Anasa* it is clear that the chromosomes of any cell maintain the typical size relations, so that particular pairs can be picked out with little trouble. The results of Rabl and Meves are the reverse of this. Foot and Strobell ('13) write: "We demonstrated in 1905 that the form and relative size of the chromosomes in *Allolobophora fœtida* are inconstant and in every publication since that date we have demonstrated variability in form, relative size and behavior of the chromosomes in every form we have studied, and we have consistently argued that such variability attacks the very foundations upon which the popular chromosome speculations of this decade have been built."

Before many of these questions can be satisfactorily answered detailed study must be made of the chromosomes in the somatic cells of many more forms, especially where the somatic and gonial numbers do not agree. Also a thorough review must be made of the many apparently aberrant cases, which have been briefly considered here.

BIBLIOGRAPHY.

Agar, W. E.

'11 The Spermatogenesis of Lepidosiren paradoxa. Quart. Journ. Micr. Sci., Vol. 57, Pt. 1.

'12 Transverse Segmentation and Internal Differentiation of Chromosomes Quart. Journ. Micr. Sci., Vol. 58.

Armbruster, L.

'13 Chromosomenverhältnisse bei der Spermatogenese solitärer Apiden. *Arch. f. Zellf.*, Bd. 11, H. 2.

Bachhuber, L. J.

'16 The Behavior of the Accessory Chromosomes and of the Chromatoid Body in the Spermatogenesis of the Rabbit. BIOL. BULL., Vol. 30, No. 4.

Barratt, J. O. W.

'07 On Mitosis in Proliferating Epithelium. Proc. Roy. Soc. London, Vol. 79 B.

Baumgartner, W. J.

'04 Some New Evidences for the Individuality of the Chromosomes. BIOL. BULL., Vol. 8, No. 1.

Bigelow, H. B.

'07 Studies on the Nuclear Cycle of Gonionemus murbachii. Bull. Mus. Comp. Zool. Harv. Coll., Vol. 48.

Boring, A. M.

'07 A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ, and the Fulgoridæ. Journ. Exp. Zool., Vol. 4, No. 4.

'13 The Chromosomes of the Cercopidæ. BIOL. BULL., Vol. 24, No. 3.

Boveri, T.

'87 Zellen-Studien. Jena.

'99 Die Entwicklung von Ascaris megalocephala, etc. Jena.

Brauer, A.

'93 Zur Kenntnis der Spermatogenese von Ascaris megalocephala. Arch. f. mikr. Anat., Bd. 42.

Conklin, E. G.

'12 Cell Size and Nuclear Size. Journ. Exp. Zool., Vol. 12, No. 1.

Davis, H. S.

'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. Mus. Comp. Zool. Harv. Coll., Vol. 53, No. 2.

Dederer, P. H.

'07 Spermatogenesis in Philosamia cynthia. BIOL. BULL., Vol. 13, No. 2.

'15 Oögenesis in Philosamia cynthia. Journ. Morph., Vol. 26, No. 1.

Della Valle, P.

'09 L'Organizzazione della Cromatina studiata mediante il Numero dei Cromosomi. Arch. Zool., 4.

Doncaster, L.

'07 Gametogenesis and Fertilization in Nematus ribesii. Quart. Journ. Micr. Sci., Vol. 51, No. 201.

Duesberg, J.

'06 Sur le Nombre des Chromosomes chez l'Homme. Anat. Anz., Bd. 28.

Farmer, J. B., and Moore, J. E. S.

'05 On the Maiotic Phase (Reduction Divisions) in Animals and Plants. Quart. Journ. Micr. Sci., Vol. 48, No. 192.

Farmer, J. B., Moore, J. E. S., and Walker, C. E.

'05 On the Cytology of Malignant Growths. Proc. Roy. Soc. London, Vol. 77 B, No. B 518.

Fick, R.

'93 Ueber die Reifung und Befruchtung des Axolotleies. Zeit. wiss. Zool., Bd. 56, H. 4.

Flemming.

'98 Ueber die Chromosomenzahl beim Menschen. Anat. Anz., Bd. 14.

Foot, K., and Strobell, E. C.

'12 A Study of Chromosomes and Chromatin Nucleoli in Euschistus crassus. Arch. f. Zellf., Bd. 9, H. 1.

'13 Preliminary Note on the Results of Crossing Two Hemipterous Species with Reference to the Inheritance of an Exclusively Male Character and Its Bearing on Modern Chromosome Theories. BIOL. BULL., Vol. 24, No. 3.

Fries, W.

'09 Die Entwicklung der Chromosomen in Ei von Branchipus, etc. Arch. f. Zellf., Bd. 4, H. 1.

Gerould, J. H.

'06 The Development of Phascolosoma. Zool. Jahrb., Bd. 23, H. 1.

Goldschmidt, R.

'05 Eireifung, Befruchtung, und Embryonalentwicklung des Zoogonus mirus. Zool. Jahrb., Bd. 21, H. 4.

'08 Die Chromatinreifung der Geschlechtszellen des Zoogonus mirus, etc. Arch. f. Zellf., Bd. 2, H. 2.

Granata, L.

* '09 Le Division degli Spermatociti di Xylocopa violacea. Biologica, Vol. 2, No. 15.

'10 Le Cinesi Spermatogenetiche di Pamphagus marmoratus. Arch. f. Zellf., Bd. 5, H. 2.

Grégoire, V.

'09 La Reduction dans le Zoogonus mirus Lss. et le "Primärtypus." La Cellule, T. 25, 2d fasc.

Grégoire, V., and Denton, W.

'06 Contribution a l'Etude de la Spermatogénése dans l'Ophryotrocha puerilis. La Cellule, T. 23, 2d fasc.

Gross, J.

'07 Die Spermatogenese von Pyrrhocoris apterus. Zool. Jahrb., Bd. 23, H. 2.

Gulick, A.

'11 Über die Geschlechtschromosomen bei einigen Nematoden, nebst Bemerkungen über die Bedeutung dieser Chromosomen. Arch. f. Zellf., Bd. 6, H. 3.

Gutherz, S.

'07 Zur Kenntnis der Heterochromosomen. Arch. f. mikr. Anat., Bd. 69.

Guyer, M. F.

'09 The Spermatogenesis of the Domestic Chicken. Anat. Anz., Bd. 34, Nos. 22–24.

'10 Accessory Chromosomes in Man. BIOL. BULL., Vol. 19, No. 4.

'14 A Note on the Accessory Chromosomes of Man. Science, N. S., Vol. 39, No. 1011.

Henking, H.

'92 Untersuchungen über die ersten Entwicklungsvorgängen in den Eiern der Insekten. Zeit. f. wiss. Zoll., Bd. 54, H. 1–2.

Hoy, W. E., Jr.

'14 A Preliminary Account of the Chromosomes in the Embryos of Anasa tristis and Diabrotica vittata. BIOL. BULL., Vol. 27, No. 1.

Jenkinson, J. W.

'04 Observations on the Maturation and Fertilization of the egg of the Axolotl. Quart. Journ. Micr. Sci., Vol. 48, No. 191.

Jordan, H. E.

'08 The Spermatogenesis of Aplopus mayeri. Pub. Carn. Inst. Wash., 102.

'11 The Spermatogenesis of the Opossum (Didelphis virginiana) with Special Reference to the Accessory Chromosome and the Chondriosomes. Arch. f. Zellf., Bd. 7, H. 1.

'14 The Spermatogenesis of the Mongoose; and a Further Comparative Study of Mammalian Spermatogenesis, with Special Reference to Sex Chromosomes. Pub. Carn. Inst. Wash., 182.

Kölliker, A.

* '89 Geweblehre des Menschen. 6te Aufl.

Korschelt, E.

'95 Über Kerntheilung, Eireifung und Befruchtung bei Ophryotrocha puerilis. Zeit. f. wiss. Zool., Bd. 60, H. 4.

Krimmel, O.

'10 Chromosomenverhältnisse in generativen und somatischen Mitosen bei Diaptomus coeruleus, etc. Zool. Anz., Bd. 35.

Lefevre, G., and McGill, C.

'08 The Chromosomes of Anasa tristis and Anax junius. Am. Journ. Anat., Vol. 7, No. 4.

Mack, J. B.

'14 A Study of the Dimensions of the Chromosomes of the Somatic Cells of Amblystoma. Kan. Univ. Sci. Bull., Vol. 9, No. 10.

Metz, C. W.

'14 Chromosome Studies in the Diptera. I. Journ. Exp. Zool., Vol. 17, No. 1.

'16 Chromosome Studies in the Diptera. II. Journ. Exp. Zool.,Vol. 21, No. 2.

Meves, F.

'97 Ueber die Entwicklung der Männlichen Geschlechtszellen von Salamandra maculosa. Arch. f. mikr. Anat., Bd. 48.

'07 Die Spermatocytenteilungen bei der Honigbiene (Apis mellifica L.) nebst Bemerkungen über Chromatinreduktion. Arch. f. mikr. Anat., Bd. 70.

'11 Chromosomenlängen bei Salamandra, nebst Bemerkungen zur Individualitätstheorie der Chromosomen. Arch. f. mikr. Anat., Bd. 77, Ab. 2.

Mohr, O. L.

'15 Sind die Heterochromosomen wahre Chromosomen? Untersuchungen über ihr Verhalten in der Ovogenese von Leptophyes punctatissima. Arch. f. Zellf., Bd. 14, H. 2.

Montgomery, T. H.

'00 The Spermatogenesis of Peripatus (Peripatopsis) balfourii up to the Formation of the Spermatid. Zool. Jahr., Bd. 14, H. 2.

'06 Chromosomes in the Spermatogenesis of the Hemiptera heteroptera. Trans. Am. Philos. Soc., Vol. 21, Pt. 3.

'10 On the Dimegalous Sperm and Chromosomal Variation of Euschistus, with Reference to Chromosomal Continuity. Arch. f. Zellf., Bd. 5, H. 1.

'12 Human Spermatogenesis. Journ. Acad. Nat. Sci. Phila., Vol. 15, 2d series.

Moore, J. E. S., and Arnold, E.

'06 On the Existence of Permanent Forms among the Chromosomes of the First

Maiotic Division in Certain Animals. Proc. Roy. Soc. London, Vol. B 77, No. B 521.

Morgan, T. H.

'14 Has the White Man More Chromosomes than the Negro? Science, N. S., Vol. 39, No. 1014.

Morrill, C. V.

'10 The Chromosomes in the Oögenesis, Fertilization and Cleavage of Coreid Hemiptera. BIOL. BULL., Vol. 19, No. 2.

Morse, M.

'09 The Nuclear Components of the Sex Cells of Four Species of Cockroaches. Arch. f. Zellf., Bd. 3, H. 3.

Mulsow, K.

'12 Der Chromosomencyclus bei Ancyracanthus cystidicola *Rud.* Arch. f. Zellf., Bd. 9, H. 1.

Murray, J. A.

'06 Zahl und Grossenverhältnisse der Chromosomen bei Lepidosiren paradoxa. Anat. Anz., Bd. 29, Nos. 7–8.

Nachtsheim, H.

'13 Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene. Arch. f. Zellf., Bd. 11, H. 2.

Payne, F.

'08 On the Sexual Differences of the Chromosome Groups in Galgulus oculatus. BIOL. BULL., Vol. 14, No. 5.

'09 Some New Types of Chromosome Distribution and their Relation to Sex. BIOL. BULL., Vol. 16, No. 3.

'10 The Chromosomes of Acholla multispinosa. BIOL. BULL., Vol. 18, No. 4.

Petrunkewitsch, A.

'01 Die Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienenei. Zool. Jahrb., Bd. 14, H. 4.

Popoff, M.

'07 Eibildung bei Paludina vivipara, etc. Arch. f. mikr. Anat., Bd. 70.

Rabl, C.

* '06 Über organbildende Substanzen und ihre Bedeutung für die Vererbung. Antrittsvorlesung. Leipzig.

Randolph, H.

'08 On the Spermatogenesis of the Earwig, Anisolabis maritima. BIOL. BULL., Vol. 15, No. 2.

Rath, O., vom.

'93 Beiträge zur Kenntnis der Spermatogenese von Salamandra maculosa. I. Zeit. f. wiss. Zool., Bd. 57, H. 1.

'94 Ueber die Konstanz der Chromosomenzahl bei Tieren. BIOL. CENTR., Bd. 14, Nr. 13.

Robertson, W. R. B.

'16 Chromosome Studies. I. Journ. Morph., Vol. 27, No. 2.

Schleip, W.

'11 Das Verhalten des Chromatins bei Angiostomum (Rhabdonema) nigrovenosum. Arch. f. Zellf., Bd. 7, H. 1.

Seiler, J.

'14 Das Verhalten der Geschlechtschromosomen bei Lepidopteren. Arch. f. Zellf., Bd. 13, H. 2.

Stevens, N. M.

'04 On the Germ Cells and the Embryology of Planaria simplissima. Proc. Acad. Nat. Sci. Phila., Vol. 56, Pt. 1.

'05 Studies in Spermatogenesis. Pub. Carn. Inst. Wash., 36.

'06 Studies in Spermatogenesis. II. Ibid.

'08a A Study of the Germ Cells of Certain Diptera, with Reference to the Heterochromosomes and the Phenomena of Synapsis. Journ. Exp. Zool., Vol. 5, No. 3.

'08b The Chromosomes in Diabrotica vittata, Diabrotica soror and Diabrotica 12-punctata. Journ. Exp. Zool., Vol. 5, No. 4.

'09 Further Studies on the Chromosomes of the Coleoptera. Journ. Exp. Zool., Vol. 6, No. 1.

'10 The Chromosomes in the Germ Cells of Culex. Journ. Exp. Zool., Vol. 8, No. 2.

'11a Further Studies on Heterochromosomes in Mosquitoes. Biol. Bull., Vol. 20, No. 2.

'11b Heterochromosomes in the Guinea-Pig. Biol. Bull., Vol. 21, No. 3.

Taylor, M.

'14 The Chromosome Complex of Culex pipiens. Quart. Journ. Micr. Sci., Vol. 60, Pt. 3.

Wasserman, F.

'13 Die Oogenese des Zoogonus mirus. Arch. f. mikr. Anat., Bd. 83, Abt. 2.

Wassilief, A.

'07 Die Spermatogenese von Blatta germanica. Arch. f. mikr. Anat., Bd. 70.

Wieman, H. L.

'13 Chromosomes in Man. Am. Journ. Anat., Vol. 14, No. 4.

Wilcox, E. V.

'00 Human Spermatogenesis. Anat. Anz., Bd. 17.

Wilson, E. B.

'05a Studies on Chromosomes. I. Journ. Exp. Zool., Vol. 2, No. 3.

'05b Studies on Chromosomes. II. Journ. Exp. Zool., Vol. 2, No. 4.

'06 Studies on Chromosomes. III. Journ. Exp. Zool., Vol. 3, No. 1.

'09a Studies on Chromosomes. IV. Journ. Exp. Zool., Vol. 6, No. 1.

'09b Studies on Chromosomes. V. Journ. Exp. Zool., Vol. 6, No. 2.

'09c The Female Chromosome Groups in Syromastes and Pyrrhocoris. Biol. Bull., Vol. 16, No. 4.

Winiwarter, H. von.

'00 Le corpuscule intermediaire et le nombre des chromosomes du Lapins Arch. de Biol., T. 16.

'01 Recherches sur l'Ovogenèse et l'Organogenèse de l'Ovaire des Mammifères (Lapin et Homme). Arch. de Biol., T. 17.

'12 Etudes sur la Spermatogenèse Humaine. Arch. de Biol., T. 27.

Winiwarter and Sainmont.

'09 Nouvelles Recherches sur l'Ovogenèse et l'Organogenèse de l'Ovaire de. Mammiféres (Chat). Arch. de Biol., T. 24.

Wodsedalek, J. E.

'13 Spermatogenesis of the Pig with Special Reference to the Accessory Chromosomes. Biol. Bull., Vol. 25, No. 1.

* The papers marked with an asterisk were inaccessible.

ASCARIS CANIS (WERNER) AND ASCARIS FELIS (GÖZE).

A TAXONOMIC AND A CYTOLOGICAL COMPARISON.

ARTHUR C. WALTON.

(From the Zoölogical Laboratory of Northwestern University.)

Introduction.

Even to-day systematic helminthologists are not of one opinion concerning the taxonomic relation of the *Ascaris* found in the dog to the *Ascaris* found in the cat. Thus Glaue ('08, '09), after a careful study of hundreds of individuals, maintains that they are two distinct and separate species; whereas two other workers, Schöppler and Krüger ('12), declare quite as emphatically that the differences between these two forms are not specific differences, but differences in degree of development only, and believe that these forms are merely varieties of *Ascaris mystax* (Zeder). In 1911 Edwards made a cytological study of *Ascaris felis*. The chromosomes in number and form differed greatly from those described by Marcus ('06) for *Ascaris canis*. Therefore upon a cytological basis, Edwards declared *A. canis* and *A. felis* to be distinct species. Dr. S. I. Kornhauser, while examining the germ cells of the *Ascaris* from the dog in connection with the formation of di-tetrads, was struck by the dissimilarity between his material and that studied by Marcus. On communicating with Dr. Marcus, it was found that he had obtained his material from a great variety of mammals which died at the Munich Zoölogical Gardens. At the suggestion of Dr. Kornhauser, the writer has made a careful taxonomic and cytological comparison of *A. canis* and *A. felis*. Taxonomically he is able to substantiate the work of Glaue; and cytologically, that of Edwards; and to prove that Marcus was not dealing with *A. canis* (Werner).

I here wish to express my thanks to Dr. Kornhauser for his criticism of my work and the preparation of this paper, and to Dr. Chas. Zell, of Chicago, for aid in obtaining material.

Material and Methods.

The material used was obtained from freshly killed dogs and cats. The worms were immediately removed from the intestine, placed in normal salt solution and kept at body temperature until they could be fixed. The ovaries and testes were at once stripped out on a glass plate and fixed. Hermann's fluid and Flemming's fluid (strong) were used to fix the testes, and Petrunkevitch's modification of Gilson's fluid was found to be the most satisfactory fixative for the ovaries. Sections of the testes were made 4 μ to 8 μ in thickness; those of the ovaries, 10 μ to 30 μ in thickness. In general the material was stained by a modified iron-alum-hæmatoxylin method: dilute Delafield's hæmatoxylin being used in the place of $\frac{1}{2}$ per cent. aqueous solution of hæmatoxylin after the mordant. This method gave better contrast between the chromatin matter and the yolk granules than could be obtained by the ordinary Heidenhain method. Orange *G* and Bordeaux Red were used as counter stains.

Taxonomic Comparison of A. canis and A. felis.

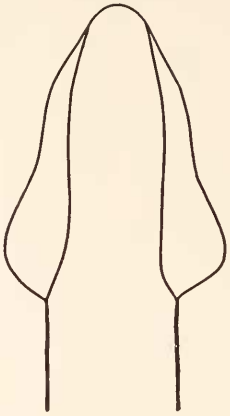
Anatomical study of one hundred specimens of *A. canis* and fifty specimens of *A. felis* gave the results shown in the following table. The table at the same time gives a comparison of the main points of difference between the two forms.

	A. canis.	*A. felis.*
Length of animal	60–120 mm.	45–95 mm.
Length of oral wing	1,950–2,250 mm.	1,450–1,720 mm.
Breadth of oral wing	350–500 mm.	400–600 mm.
Shape of oral wing	Lanceolate	Cordate.
Cross-section, oral wing	Chitinous rod, long and narrow.	Chitinous rod, broad and flat.
Wing spicules	Long, crescent-shaped	Long, semi-circular.
Form of tail of male	Gradual slope to point	Folded ventrad to point.
Postanal papillae	4 ventral, 3 dorsal pairs	3 ventral, 2 dorsal pairs.
Anal spicules	Short, narrow	Longer, broader.
Body segments	Narrow	Broader than *A. canis*.

From this comparison it is seen that *A. canis* is longer than *A. felis* and has an oral wing which is lanceolate rather than cordate in shape (Text-figs. *A* and *B*). Cross-sections of the oral wing show differences in the shape and size of the supporting

chitinous rods of the two forms (Text-figs. *C* and *D*). The number and relative positions of the postanal papillæ in *A. canis*

TEXT-FIG. *A*.



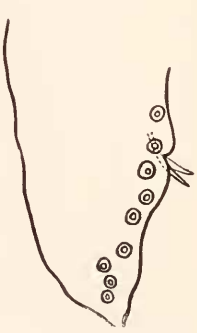
TEXT-FIG. *B*.

differ from those of *A. felis* (Text-figs. *E* and *F*). These results substantiate those recorded by Glaue ('08 and '09), and are of

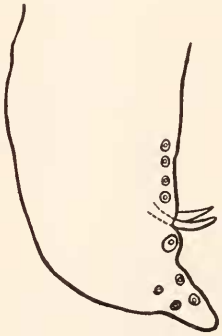
TEXT-FIG. *C*.

TEXT-FIG. *D*.

too constant and specific a character to be classed as variations of the same species, as claimed by Schöppler and Krüger ('12).



TEXT-FIG. *E*.



TEXT-FIG. *F*.

In the specimens examined by the writer there were none of the variations claimed by the writers last mentioned, and therefore the present writer maintains upon a morphological basis that the *Ascaris* of the dog is a species distinct from the *Ascaris* of the cat.

Spermatogenesis of A. canis and A. felis.

In the present article only very characteristic stages in the spermatogenesis will be considered.

1. *A. canis.*

The chromatin of the spermatocytes nearing the end of the growth period appears in the form of two irregular masses situated peripherally in the nucleus. One mass is small and circular, whereas the other is long and narrow, lying within the nuclear membrane and extending along half of its circumference (Plate I., Fig. 1). There is also a plasmosome which loses its affinity for stains and disappears shortly after the formation of the prophase chromosomes. The two chromatin masses early assume a vacuolated appearance and become distinct chromosomes. From the larger chromatic mass are formed twelve large chromosomes; and from the smaller karyosome are formed six small chromosomes, closely grouped. Thus we find early that the haploid number of chromosomes, eighteen, clearly defines itself. The group of twelve large chromosomes retains a peripheral position during the prophase, while the smaller group migrates early to the central part of the nucleus.

The centrosome is of extranuclear origin as it sets up the mitotic figure before the nuclear membrane disappears (Plate I., Fig. 2). The spindle fibers seem to arise from the centrosome and push against the nuclear membrane, which often shows irregular indentations in the region of the centrosome.

In the metaphase of the first spermatocyte division the chromosomes are arranged in the equatorial plate, with the group of six chromosomes in the middle and the twelve larger chromosomes scattered more peripherally (Plate I., Fig. 3). From a polar view these peripheral chromosomes all show a distinct tetrad form. From a lateral view, careful study reveals that the twelve large chromosomes also show a tetrad form (Plate I.,

Fig. 4), indicating that the chromosomes of the first maturation spindle are really di-tetrads, or octads, due to the presence of a "Querkerbe" in each of the four components. The number of chromosomes is easily seen to be eighteen (Plate I., Fig. 3).

The anaphase of the first spermatocyte division shows that the group of six small chromosomes lags behind (Plate I., Figs. 5–7), and goes undivided to one pole, thus constituting a heterochromosome group of the X-type, and in the late anaphase this group goes to one of the daughter plates (Fig. 7); thus forming two types of spermatocytes, one having eighteen and the other only twelve chromosomes. Polar views of the telophases of this division show that one plate receives half of the autosome material and all of the idiosome material, and that the other daughter plate receives only one half of the autosome material (Plate I., Figs. 8*a* and 8*b*). Sister second spermatocytes in the metaphase show very clearly the difference in the number of chromosomes found in the two types of second spermatocytes (Plate I., Fig. 9). Lateral views of the same stage show the size difference in the equatorial plate (Plate I., Fig. 10*a* with 18 chromosomes, Fig. 10*b* with 12 chromosomes).

The second division of the spermatocytes is entirely regular, each spermatid receiving one half the amount of chromatic material. In lateral views of the telophase of the second division the daughter plates from an eighteen chromosome second spermatocyte are larger than those from a twelve chromosome second spermatocyte (Plate I., Figs. 11*a* and 11*b*). Two types of spermatids can be recognized, those with larger nuclei and those with smaller nuclei. The chromosomes at this stage have become so diffused that they can no longer be distinguished as individual bodies (Plate I., Fig. 12*a*, eighteen chromosomes; Fig. 12*b*, twelve chromosomes).

2. *A. felis.*

Polar views of the metaphase plates of the first spermatocyte division show nine tetrad chromosomes, one of which is asymmetrical and larger than any of the remaining eight. This large tetrad is composed of two unequal parts, the larger component being as large as one of the ordinary tetrads. The smaller

component is about half the size of the autosome tetrads. The first division is transverse, and separates the unequal components of the large tetrad, or hexad, which is doubtlessly to be classed as a sex-chromosome, either of the XY-type or as an X-chromosome attached to the end of an autosome tetrad.

In the first division the one daughter plate receives eight diad autosomes and the larger component of the heterochromosome, and the other plate receives eight autosome diads and the smaller component of the heterochromosome. The second spermatocyte division is equational and entirely regular, each spermatid receiving nine chromosomes. As there are two types of second spermatocytes, there are also two types of spermatids formed, one having nine chromosomes all of the same size, and the other having nine chromosomes, one of which is larger than the other eight.

These results agree with those obtained by Edwards ('11) in his work on *A. felis*, and indicate that in all probability the form studied by the writer and by Edwards are the same form of *A. felis*. Since the figures made from my slides are in all respects similar to those published by Edwards, it was thought unnecessary to reproduce them here.

Discussion.

The present work shows *A. canis* different both morphologically and cytologically from *A. felis*. It shows also that the Nematodes worked upon by Marcus were evidently not *A. canis*. Marcus did not offer any taxonomic evidence that he was dealing with *A. canis*, this being merely another case in which a cytologist has not given proper consideration to the systematic standing of his material. The spermatocytes in the Nematodes described in Marcus's work contained twelve chromosomes, ten bivalents and two univalents, and no heterochromosome group. As errors in observation can not account for such diversity, it is doubtless due to differences in material.

Summary.

The following are the most important points brought out in this study:

1. Material used is same as recognized European forms of *Ascaris canis* (Werner) and *Ascaris felis* (Göze).

2. *A. canis* shows 18 chromosomes for the haploid number, 12 di-tetrad autosomes and 6 tetrad idiosomes. These 6 tetrad idiosomes form a heterosome group of the X-type.

3. There are two types of second spermatocytes and spermatids found, one type having 12 autosomes and 6 idiosomes, the other type having but the 12 autosomes.

4. In *A. felis* there are 9 chromosomes in the primary spermatocytes, 8 autosomes and one large heterochromosome composed of unequal parts. This is in agreement with Edwards.

5. *A. canis* and *A. felis* are morphologically and cytologically two different species, not varieties of the same species.

6. Chromosomes of true *A. canis* do not agree in form or number with those described by Marcus for his "so-called" *A. canis*.

BIBLIOGRAPHY.

Boveri, Th.

'09 Ueber Geschlechtschromosomen bei Nematoden. Arch. f. Zellf., Bd. IV., Hft. 1, pp. 132–141. 2 Textfiguren.

Edwards, C. L.

'11 The Sex-Chromosomes in Ascaris felis. Arch. f. Zellf., Bd. VII., Hft. 3, pp. 309–313. Plate XVII.

Glaue, H.

'08 Zur Unterscheidung von Ascaris canis und A. felis (Ascaris canis s. mystax). Zool. Anz., Bd. XXXIII., Nr. 24, 25, S. 785–790. 3 Textfiguren.

'09 Beitraege zu einer Monographie der Nematoden species Ascaris felis und Ascaris canis. Zeitschr. wiss. Zool., Bd. 95, Hft. 3, S. 551–593. 26 Textfiguren.

Marcus, H.

'06 Ei und Samenreife bei Ascaris canis (Werner) (Asc. mystax). Arch. Mik. Anat., Bd. LXVIII., Hft. 3, S. 441–491. Taf. XXIX–XXX. 10 Textfiguren.

Schneider, A.

'66 Monographie der Nematoden. S. 34–39. 1 Textfigur.

Schoeppler, H., and Krueger, P.

'12 Zur Unterscheidungsfrage von Ascaris canis und Ascaris felis (A. canis s. mystax). Centralbl. Bakt. Parasit., Abt. 1, Orig. Bd. LXII., S. 143–146.

EXPLANATION OF PLATES.

All figures were made with the aid of a camera lucida, at a magnification of 2,800 diameters; Spencer apochromatic objective 2 mm., compensating ocular 20 × and projection distance 310 mm.

FIG. 1. Prophase of first spermatocyte, showing formation of chromosomes and separate grouping of heterochromosomes. The plasmosome is just above heterochromosome group.

FIG. 2. Late prophase, first spermatocyte, spindle formed but nuclear membrane still intact.

FIG. 3. Metaphase plate, first spermatocyte, showing heterochromosome group in center; number of chromosomes, 18.

FIG. 4. Metaphase plate, first spermatocyte, lateral view showing breaking down of nuclear membrane.

FIG. 5. Early anaphase, first spermatocyte, optical section through equatorial plate, showing lagging heterochromosome group.

FIG. 6. Late anaphase, first spermatocyte, showing lagging heterochromosome group of six components.

FIG. 7. Late anaphase, first spermatocyte, heterochromosome group going to upper daughter cell. Central spindle degenerated.

FIG. 8*a*. Polar view of daughter plate of first division receiving heterochromosome group; number of chromosomes, 18.

FIG. 8*b*. Polar view of daughter plate of first division not receiving heterochromosome group; number of chromosomes, 12.

FIG. 9. Metaphase plates of two sister second spermatocytes, which have not separated. One plate contains 18, the other 12 chromosomes.

FIG. 10*a*. Metaphase plate, second spermatocyte, lateral view of plate containing 18 chromosomes, *i. e.*, containing heterochromosome group of 6 and 12 autosomes.

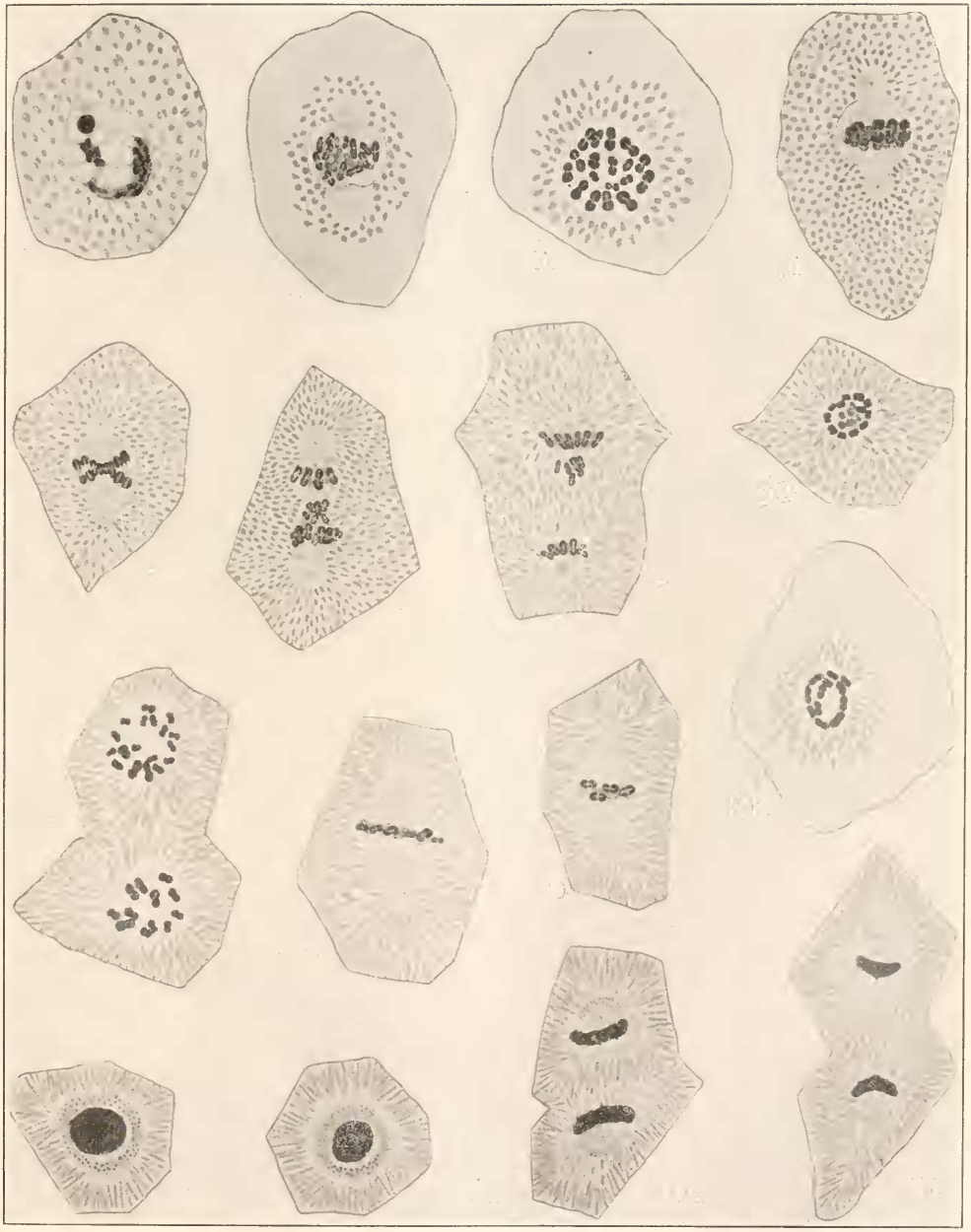
FIG. 10*b*. Metaphase plate, second spermatocyte, lateral view of plate containing 12 chromosomes, *i. e.*, lacking the heterochromosome group of six.

FIG. 11*a*. Telophase of second spermatocyte division, having 18 chromosomes.

FIG. 11*b*. Telophase of second spermatocyte division, having 12 chromosomes.

FIG. 12*a*. Spermatid having 18 chromosomes.

FIG. 12*b*. Spermatid having 12 chromosomes.



A. C. W. DEL.

A Taxonomic and a Cytological Comparison of Ascaris canis (Werner) and Ascaris felis (Göze).

ON THE OCCURRENCE OF A PARASITE OF THE PIKE IN EUROPE, *MYXIDIUM LIEBERKÜHNI* BÜTSCHLI, IN THE PIKE ON THE AMERICAN CONTINENT AND ITS SIGNIFICANCE.

JAMES W. MAVOR,
UNIVERSITY OF WISCONSIN.

CONTENTS.

1. INTRODUCTION.

In a previous paper (Mavor, 1916*b*) the author has recorded the occurrence of what he believed to be *Myxidium lieberkühni* Bütschli in the urinary bladder of the Pike, *Lucius lucius* L. from the Georgian Bay. The parasite was then identified on the basis of the structure of the plasmodial stage, and two spores which were all the author was able to find at that season of the year, July.

While searching for the parasite in a pike caught in Lake Mendota, Wisconsin, on May 5, 1916, he was able to find abundant spores and stages in the sporogenesis. This material forms the basis of the present paper.

2. FINDINGS IN FRESH PREPARATIONS.

The plasmodial stage was very abundant in the urinary bladder. When this was examined the urine had been evacuated and what little remained upon being withdrawn with a pipette held close to the inner surface of the bladder was filled with plasmodia. These with the exception of a few larger forms were almost uniformly spherical in shape and ranged from 10 μ to 80 μ in diameter. Many of these show areas on their surfaces

where tufts of fine short pseudopodia come off, a condition which was found even in the smallest of the pseudopodia, Pl. I., Fig. 1. In all the plasmodia a clear differentiation into ectoplasm and endoplasm could be seen, but evidence of the mesoplasm was not found, which was also the case in the parasite recorded from the Georgian Bay (Mavor, 1916b). The yellowish globules indicated by clear circles (Pl. I., Figs. 1, 3, 4) showed their probably oily nature by flowing together in plasmodia which had remained in the excised and decomposing urinary bladder for about 40 hours. Crystals of a hæmatoid nature were of rather rare occurrence and were found only in the smaller plasmodia and of these usually only in those not containing spores.

The *spores* occur in the plasmodia in pairs and lay so that their concave surfaces are in juxtaposition (Pl. I., Figs. 2 and 4). The shape of the spores of this species has been described by Bütschli (1882), Thelohan (1895), Cohn (1896), and Mavor (1916*b*). It is that of a spindle slightly curved so that when viewed from the side (Pl. I., Fig. 2), the spore appears crescent-shaped. The surface of the spore is marked with longitudinal striations converging toward the ends. The polar capsules are situated at either end of the spindle and each occupies rather less than one third of the length of the spore. In the preparations studied by the author, the polar filaments can be easily seen within the capsules in the fresh state as a spiral of four or five coils. They were extruded under the action of concentrated sulphuric acid but remained in the capsules when treated with a solution of iodine in potassic iodide and when treated with ammonia water. The sporoplasm occupies the central portion of the spore and contains two or more highly refractive bodies thought to be nuclei. The average dimensions of ten spores were found to be:

Length	18–19 μ
Width	5–6 μ
Length of Polar Capsules	5 μ
Width of Polar Capsules	2.5–3.0 μ
Length of polar filaments	40–45 μ

3. Findings in Stained Preparations.

The material was fixed in hot Schaudinn's fluid and stained with either borax-carmine, Delafield's hæmatoxylin, or Giemsa's stain. For the method used in applying the latter see Mavor (1916*a*).

The myxosporidia were found to contain nuclei of two sizes; larger nuclei measuring 2.5 μ in diameter and smaller nuclei 1.2 μ in diameter. No essential differences of structure were observed in these nuclei and no difference could be observed in their reaction to Giemsa's stain as was found by the author to be the case in the nuclei of *Ceratomyxa acadensis* (Mavor, 1916*a*). There are two kinds of granules evident in preparations stained with Giemsa's stain and they show the same reactions as described by the author in his previous paper (Mavor, 1916*b*).

The sporogenesis follows in its later stages the method described for *Myxobolus pfeifferi* by Keysselitz (1908). The sporoplasm of the fully formed spore contains two nuclei.

4. The Significance of the Occurrence of Myxidium Lieberkühni in America.

There can be little doubt that the myxosporidia of fishes are without an intermediate host. Their life-cycle consists of a period spent in the body of the host-fish alternating with a period during which the spores are free in the water or are passing unaffected through the digestive tract of some other aquatic animal. There are three ways in which their geographical distribution may be extended; (1) the spores may be carried in currents of water, (2) the spores may be carried in the digestive tract of aquatic animals, (3) all stages may be carried by the host-fish and accompany it in its wanderings. That the spores could be carried from the fresh water of one continent to the fresh water of another continent by either of the first two methods seems unlikely. It would seem therefore probable that *Myxidium lieberkühni* has followed in its distribution the wanderings of its host *Lucius lucius*.

Lucius lucius is a very old species. "Remains of the common pike occur in abundance in quaternary deposits" (Gunther, 1880, p. 624). Furthermore, not only the genus but the family,

Esocidæ, is known only from fresh water. "Fossil pike, belonging to the existing genus, have been found in the fresh water chalk of Oenigen and the diluvial marl of Silesia" (Gunther, 1880, p. 624).

If then the distribution of *Myxidium lieberkühni* over both Europe and America dates from the time when *Lucius lucius* attained that distribution it too must be an old species, and like its host have remained unmodified through a long period.

A somewhat parallel condition is found in the *Mallophaga*, the insect parasites of birds, where a very close relation exists between parasite and host. "But it is to be noted that in practically all the cases of the common occurrence of a Mallophagan species on two or more host-species, whether these host species are of the same or neighboring regions or restricted to different continents where this commonness cannot be explained by the possibility of a meeting and actual contact of individuals of the different host-species, the distinct host-species are closely allied, that is, are usually both of the same genus. And I believe that the explanation of this condition is that the Mallophagan species has persisted unchanged on two or more diverging host-species from their common ancestor. In ancient times, geographical races arose within the limits of the ancestral host-species; these races or varieties have now come to be distinct species, distinguished by superficial differences in color and markings of plumage, etc. But the parasites of the ancient hosts have remained unchanged; the plumage as food, the temperature of the body, practically the whole environment of the insects, have remained the same; there has been no external factor at work tending to modify the parasitic species, and it exists today in its ancient form, common to the newly arisen descendants of the ancient host" (Vernon L. Kellogg, 1908, p. 3).

PAPERS CITED.

Bütschli.

'82 *Myxidium Lieberkühni* in Bronn's Kl. Ordn. Bd. 1, p. 593.

Cohn, L.

'96 Über die Myxosporidien von *Esox lucius* und *Perca fluviatilis*. Zool. Jahrb. Abt. f. Morph. Bd. 9, p. 227.

Kellogg, Vernon L.

'08 *Mallophaga* in Genera Insectorum. 66me fascicule.

Keysselitz, G.

'08 Die Entwicklung von *Myxobolus pfeifferi*. Arch. f. Protist. Bd. 11, p. 252.

Mavor, J. W.

'16a On the Life-History of *Ceratomyxa acadiensis*, a new species of Myxosporidien from the Eastern Coast of Canada. Proc. Amer. Acad. Arts and Sciences, Vol. 51, No. 11.

'16b Studies on the Protozoan Parasites of the Fishes of the Georgian Bay. Trans. Roy. Soc. Canada, Sec. IV, p. 63.

Thélohan, P.

'95 Recherches sur les myxosporidies. Bull. Scient. France et Belgique. Paris, T. 26, p. 100.

EXPLANATION OF PLATE.

PLATE I.

Myxidium lieberkühni Butschli from the urinary bladder of *Lucius lucius*. All drawings made with the camera lucida from fresh preparations of the urine.

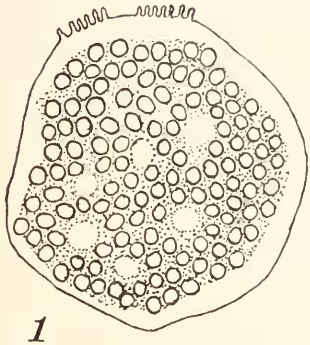
FIG. 1. Plasmodial stage showing fine pseudopodia for attachment to bladder wall. × 880.

FIG. 2. Two spores as arranged in pansporoblast. × 4,000.

FIG. 3. Small plasmodial stage showing hæmatoidin crystal. × 880.

FIG. 4. Plasmodial stage showing two pansporoblasts each containing two spores. × 880.

FIG. 5. Single spore showing sporoplasm and polar filaments. × 4,000.



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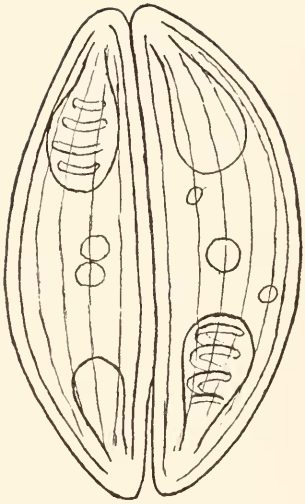

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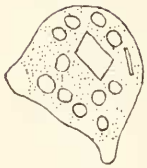

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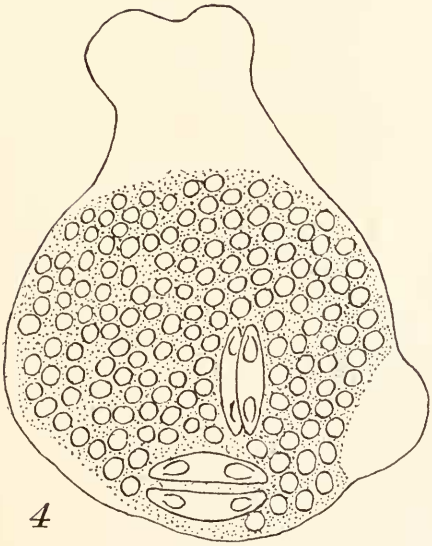

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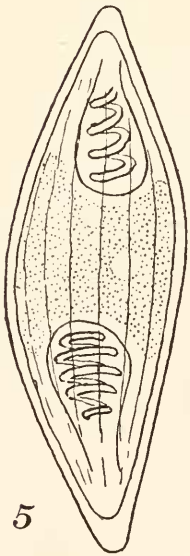

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BIOLOGICAL BULLETIN

EFFECTS OF ACUTE ALCOHOLIZATION ON THE GERM CELLS OF FUNDULUS HETEROCLITUS.

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CONTENTS.

INTRODUCTION.

This paper presents a study of the effects of short length treatments of the germ cells of *Fundulus heteroclitus* with various

concentrations of alcohol. The problem was at first undertaken with the idea of determining what might be the results of a direct treatment of the spermatozoa with alcohol. Early in the progress of the work, it became apparent that even in low concentrations the solutions used were markedly toxic to the unfertilized egg. There seems no practical way to separate the spermatozoa from the alcohol with which they are treated, and some of this material must come in contact with the eggs in the process of fertilizing them. So, for comparative purposes, an extended study of the susceptibility to acute alcoholization in the eggs of *Fundulus* prior and subsequent to fertilization was made. This indicates that the periods before and shortly after fertilization are especially critical in the history of the egg, a fact which, if it holds true generally, is of considerable significance and interest. The latter portion of the paper deals with the treatment of the spermatozoa of *Fundulus*, and the results of this on the subsequent development in the eggs fertilized by them.

There are many advantages in favor of the method of treating the germ cells directly with alcohol; for here the problem is not one involving the secondary effects of the altered soma upon the germ plasm. The fish *Fundulus* provided what seemed excellent material for such direct experimentation. The short-lived nature of the sperm of this species is, however, a fatal obstacle to anything like an extended treatment of the male germ cell. On the other hand, if the eggs of this form are allowed to stand for a much greater period than one half hour after being stripped, the percentage of fertilizations is materially lowered and in some instances the cleavages rendered abnormal. Thus these two conditions necessarily make the period of treatment a short one. Since practically no work of this kind has been done on short length treatments with different chemical poisons, this feature alone of these experiments would seem to make them of value.

Much interest has recently centered in the treatment of the germ cells of a number of different types of animals and the resulting effect on the processes of development. Notable in this regard are the experiments of Stockard ('12, '13, '16) showing the effects of alcohol on the germ cells of mammals, and those of the Hertwigs ('11, '12, '13) on the effects of radium on some less

highly developed types. Reviews of the literature along this line are readily accessible; so only the immediately applicable part need be considered here.

Dungay ('13) has made a study of the effects of heat, alcohol, alkali and hydrochloric acid upon the spermatozoa of *Nereis* and *Arbacia*. He finds that all of these agencies have an injurious effect upon subsequent development in the eggs fertilized by the treated spermatozoa.

Oppermann ('13) has found that radium modifies the sperm of the trout. His results very closely parallel those of Oscar and Gunther Hertwig on the frog in that treatments of a more limited nature serve to very profoundly modify the normal processes of development, and to produce many abnormalities. More extended treatment acted to so completely alter the spermatozoön that it retained only its fertilizing power, the eggs developing by a type of parthenogenesis.

In the same year Gunther and Paula Hertwig ('13) report that by treating the spermatozoa of Gobius jozo with 0.02 per cent. and 0.1 per cent. methylen blue for an hour, marked abnormalities occur in the development of normal eggs fertilized by spermatozoa thus modified. However, treatment of the spermatozoa of the same species for forty-five minutes with 0.1 per cent. solution of methylen blue showed no effect on these spermatozoa when used to fertilize the eggs of another fish, *Crenilabrus pavo*. This last fact makes the results obtained seem somewhat contradictory in nature.

The experiments reported in this paper were performed at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1915 and 1916, and the writer wishes to express his appreciation to the director, Professor F. R. Lillie, and the authorities there for the facilities afforded him for the work. It is a pleasure, too, to acknowledge his indebtedness to Professor Charles R. Stockard for the suggestion of the problem and much helpful interest in the progress of the work.

Material and Methods.

The grades of alcohol used were dilutions of absolute alcohol of the best quality obtainable at the time. About half of the

material used was Kahlbaum's; the other was Eimer and Amend's absolute alcohol. The experiments show no differences between the effects of the two grades. The sodium hydroxide solutions used for activating the spermatozoa and for treating the eggs were dilutions of carefully titrated normal solutions.

In treating the eggs prior to fertilization, they were stripped and placed immediately in Syracuse watch glasses filled with the strengths of alcohol to be used. At the end of the period of treatment, they were washed out into finger bowls to which a quantity of sea water was twice added and decanted. Then the eggs were removed to a clean watch glass and fertilized with fresh spermatozoa, the mixture being allowed to stand for about fifteen minutes. They were then returned to a clean finger bowl of sea water. The washing lasted for a period of about five minutes, since in each series a number of lots of eggs was involved. With each experiment a control was operated, this being fertilized at the same time as the treated eggs.

In the experiments on the sperm, one tenth of a cubic centimeter of the grade of alcohol used was measured from a pipette into a Syracuse watch glass. A male *Fundulus* which had been carefully dried in a towel to remove all water from the surface of the body, was stripped and the drop of milt pressed into the fluid in the dish. The suspension was then stirred so as to render the distribution of the spermatozoa uniform. At the end of the period of treatment, the eggs to be fertilized were poured over the treated mass of spermatozoa. It was found that a high percentage of fertilizations always results in the control by leaving the eggs for five minutes in the drop of milt; so, in these experiments on the treatment of the spermatozoa, the whole series, with few exceptions, were allowed five minutes' contact with the mass of sperm cell for fertilization. In each experiment the influence of the alcohol on the egg alone was controlled by a lot of eggs treated with the same strength prior to fertilization.

Treatment of the Female Germ Cells.

1. *Prior to Fertilization.*

(*a*) *Condition of the Egg at this Period.*—At the time of laying, the egg of *Fundulus* is in many ways in a critical period of its

history. The important and delicate processes of maturation are in progress preparatory to fertilization. The character of its protective membranes has not yet been strengthened by the formation of the fertilization membrane. So, it might well be expected that profound effects may be produced at this time by what under other conditions would be considered slight cause. This seems to be quite clearly the case in the treatment of the eggs of *Fundulus* prior to fertilization.

(*b*) *Effects of Delayed Fertilization on Control Eggs.*—Upon examining the data given in the accompanying table (see Table I.) one might easily be led from the record of the control into the error that the eggs dealt with were a poor lot. The only indication of this is in the very much lowered percentage of fertilizations; for the number of sub-normal individuals, except in few instances, is slight. It seems clear, therefore, that if the eggs of *Fundulus* are allowed to stand for some twenty to thirty minutes before fertilization, because of the closure of the micropyle, or for some other reason, a number do not fertilize. These same eggs fertilized as soon as stripped give some seventy-five to ninety per cent. of fertilizations. The fact that the controls were fertilized at the same time as the treated lots serves, however, to place this factor upon the same basis in the comparison of results.

(*c*) *Effects of Treatment on the Viability of Eggs.*—In the experiments presented in Table I. and summarized in Table II., the first four series were treated for fifteen minutes prior to fertilization; the remainder were treated for twenty minutes. It will be noted that no developing individuals were secured from the eggs treated with strengths of alcohol higher than ten per cent., and only one in treatments of this percentage. These higher concentrations produced in many instances a shrinkage and distortion of shape in the egg, and of course the highest grades killed or fixed a number of the eggs.

In the case of those treated with two per cent. and five per cent. alcohol, the number of developing individuals is lowered several hundred per cent. as compared with the controls. This result is uniform throughout the whole series. In two instanecs, no individuals developed in the treatments with five per cent. alcohol.

(*d*) *Effects of Treatment on Cleavage.*—The "percentage developing beyond early cleavages" as applied in these results is a term which does not indicate the percentage of fertilizations except in the control. Here there are very few of the aberrant cleavages, though occasionally one does occur. However, in practically every series of eggs treated with two per cent. or five per cent. alcohol there occur some four or five hours after fertailiztion a number of aberrant cleavages. In some instances there may be as many as twenty or thirty of these in a single lot of eggs; in others only a very few.

The number of eggs developing in each lot treated is so small that it was rather difficult to follow the rate of cleavage as compared with the control. In the treated lot there were usually to be observed at the time of the first cleavage in the control a few in a similar stage. As will be noted from the legend accompanying the figures (see Figs. 1–9), the aberrant cleavages of the types figured in the text represent delayed cleavages in almost every instance.

In three series of experiments, all of these aberrant cleavages were removed to a separate dish. Upon examination several hours later the blastodiscs of the most of them were found to have disintegrated and to resemble the condition of the eggs that had not been fertilized at all. Frequently two or three out of some eighteen or twenty continued to develop at a slow rate, producing in most instances defective individuals or those of low vigor.

Sections were cut of several of these irregular cleavages, but aside from the fact that fragmented nuclear material of some sort seems to be present, the time available has prevented a fuller analysis. The cleavage planes are not deeply cut into the cytoplasm of the blastodisc, but are superficial in extent. It may be that the effect of the alcohol upon the egg nucleus is such that it causes fragmentation, followed by various degrees of rounding up of the adjacent cytoplasm. Other possibilities suggest themselves. The nature of these cleavages seems to afford an interesting problem, and one of which the writer hopes to make a closer study at some later date.

(*e*) *Effects on Percentage Developing Sub-normally.*—In these

TABLE I.

SHOWING EFFECTS OF TREATING EGGS WITH SEA WATER SOLUTIONS OF ALCOHOL FOR SHORT PERIODS PRIOR TO FERTILIZATION.

Percentage of Alcohol.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Six Days.	Total Number Sub-normal.	Percentage Sub-normal.
Series I.						
Control....	294	67	22.8	67	0	0
2.....	135	10	7.4	9	9	90
5.....	103	4	3.9	4	2	50
10.....	112	0	0	0	0	—
15.....	101	0	0	0	0	—
20.....	91	0	0	0	0	—
25.....	110	0	0	0	0	—
30.....	135	0	0	0	0	—
50.....	140	0	0	0	0	—
Series II.						
Control....	142	32	22.5	32	0	0
2.....	129	6	4.7	6	1	16.7
5.....	186	5	2.7	1	4	80
10.....	101	1	0.99	0	1	100
15.....	97	0	0	0	0	—
Series III.						
Control....	142	82	57.7	77	5	6.1
2.....	129	11	8.5	10	3	27.2
5.....	151	2	1.3	1	2	100
Series IV.						
Control....	126	36	28.6	33	3	8.3
2.....	108	2	1.8	0	2	100
5.....	123	0	0	0	0	—
Series V.						
Control....	163	75	46	71	4	5.3
2.....	224	8	3.5	8	3	37.5
5.....	171	0	0	0	0	—
10.....	176	0	0	0	0	—
Series VI.						
Control....	212	54	25.4	54	0	0
2.....	248	4	1.6	4	2	50
5.....	250	2	0.8	2	1	50
10.....	116	0	0	0	0	—
Series VII.						
Control....	99	40	40	38	2	5
2.....	134	5	3.7	5	3	60
5.....	123	2	1.6	1	2	100

experiments the developing eggs were separated from the undeveloping just as soon as practicable. In the treated eggs this meant during the first day, and in the control, not later than the second day. Thus it was possible to keep up with all of the eggs in the lot with accuracy. Observations were recorded in most instances twice a day in the earlier part of the experiment; and at least once a day thereafter until its conclusion. This means that those eggs which were weakened in vitality due to natural

causes and as the result of treatment are included in the percentage sub-normal recorded. Since development in this fish is well advanced at the end of the sixth day—circulation is well established by the third or fourth day, and the eyes well formed by the end of the sixth day—this is the period at which most of the experiments were terminated. This was done for practical experimental purposes, since to record observations on so large and rapidly accumulating a series would be almost an impossibility.

TABLE II.

SHOWING COMBINED RESULTS OF THE SEVERAL EXPERIMENTS ON EGGS PRIOR TO FERTILIZATION.

Percentage of Alcohol.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Six Days.	Total Number Sub-normal.	Percentage Sub-normal.
Control.....	1176	386	32.8	372	14	3.7
2.....	1107	46	4.1	42	23	50
5.....	1107	15	1.3	9	11	73.3
10.....	505	1	0.2	0	1	100

Examination of the accompanying table (see Table II.) will show that the percentage of sub-normals in the controls scarcely averages four per cent. In the dishes that were treated with two per cent. alcohol this varied from sixteen per cent. sub-normal to as high as ninety, with an average of fifty per cent. In almost every lot there were at least a few which came through their development normally. It was also frequently noted that where in earlier development every individual in a dish appeared abnormal, later on with circulation successfully established, the early deformity became regulated, and at the end of the sixth day the individual was included among the normal.

The effects of the five per cent. alcohol were more deep seated. In some instances only one individual came through to the end of the sixth day, and this was very abnormal. The types of defects produced in these treatments are considered in another part of this paper.

2. *Immediately after Fertilization.*

(*a*) *Condition of Egg at this Period.*—It has been shown by Loeb ('13) and others that the entrance of the spermatozoön in

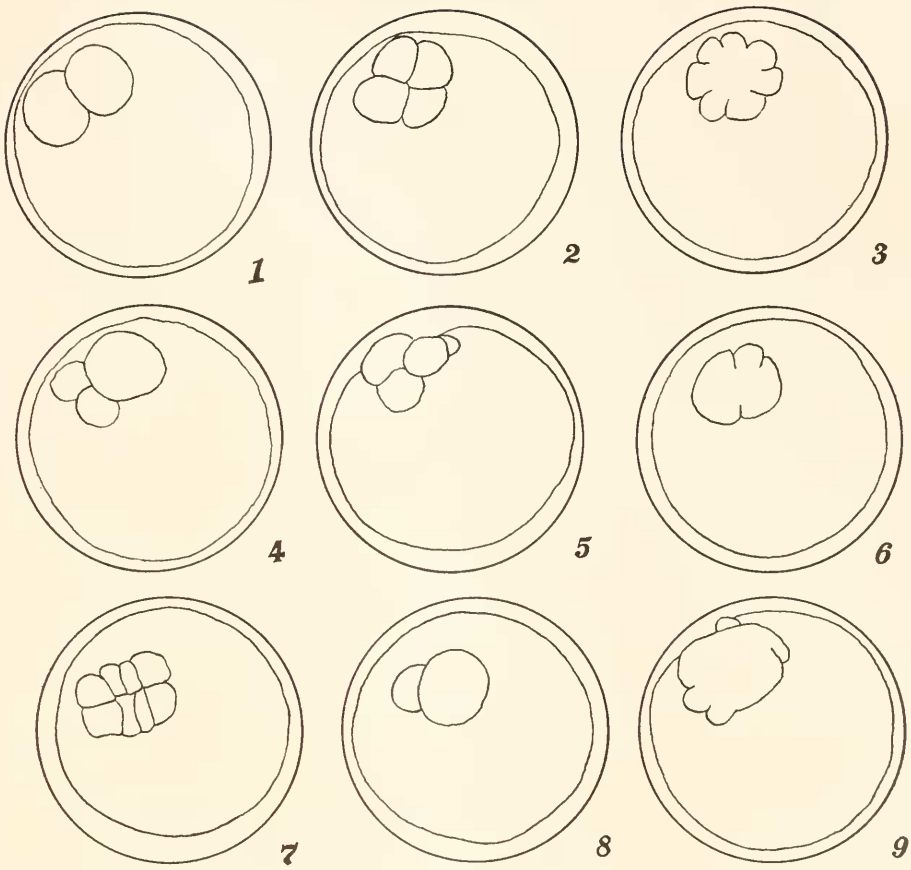


FIG. 1. A normal two-cell stage of *Fundulus* egg drawn to same scale as other cleavages.

FIG. 2. A normal four-cell stage.

FIG. 3. An aberrant cleavage occurring five hours after fertilization from egg treated with five per cent. alcohol in sea water prior to fertilization with norma sperm.

FIG. 4. An abnormal cleavage of an egg which was fertilized by sperm treated with ten per cent. alcohol in sea water.

FIG. 5. An irregular cleavage occurring three hours after fertilization in an egg which was treated with three per cent. alcohol in sea water for fifteen minutes prior to fertilization with normal sperm.

FIG. 6. An imperfect cleavage occurring four hours and a half after fertilization in an egg which was treated with two per cent. alcohol in sea water for fifteen minutes prior to fertilization with normal sperm.

FIG. 7. A normal eight-cell cleavage stage.

FIG. 8. An abnormal cleavage occurring two and a half hours after fertilization in an egg treated with two per cent. alcohol in sea water prior to fertilization with normal sperm.

FIG. 9. An aberrent cleavage occurring four hours after fertilization in an egg treated with five per cent. alcohol for twenty minutes prior to fertilization with normal sperm.

the process of fertilization instantaneously effects a marked change in the membrane of the egg. The permeability of this to certain substances seems to be increased, thus adapting the young developing individual to the interchange of materials which is necessary to its development.

There is also at this time a critical period in the nuclear phenomena. The male pronucleus has entered and is becoming acclimated to the new environment of the egg cytoplasm. The maturation divisions are being concluded and the female pronucleus preparing for union with the male pronucleus and the formation of the first cleavage spindle. These things indicate a great increase in the metabolic activity of the egg.

(*b*) *Effects of Higher Percentages on Development.*—It seemed that if the effects of treatment prior to fertilization were so decided, it would be well to test the period immediately after fertilization. Accordingly, a lot of eggs were subjected twenty minutes after fertilization to a graded series of alcohol to as high as twenty-five per cent. The results of this experiment are given in an accompanying table (see Table III.). Here it will be

TABLE III.

RESULTS OF TREATMENT OF EGGS WITH SEA-WATER SOLUTIONS OF ALCOHOL FOR FIFTEEN MINUTES AT TWENTY MINUTES AFTER FERTILIZATION.

Percentage of Alcohol.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Six Days.	Total Number Subnormal.	Percentage Subnormal.
Control	117	86	73.5	86	1	1.1
1	121	96	79.3	95	2	2
2	136	108	79.4	107	8	7.4
5	116	86	74.1	86	7	8.1
10	131	103	78.6	103	41	40
15	119	96	80.6	96	77	80
25	116	30	25.9	24	23	77

noted that the lower percentages produce an effect that is at least noticeable as compared with the control. However, it is necessary to use percentages of ten per cent. and over to secure the same effects at this period as are produced by two per cent. prior to fertilization.

The process of development is retarded by these treatments, and particularly so in the higher percentages. In the first

cleavage stage, only a small percentage were observed to be cleaving in these when the control showed a large proportion. It will be noted though that in all of the dishes except the one treated with twenty-five per cent. alcohol the percentage developing is as good as that of the control. A number of eggs were prevented from developing at all by treatments of this higher strength.

The types of defects secured by these short length treatments are much the same as those reported by Stockard ('10) from longer treatments of the eggs with lower percentages and beginning in the early cleavages. This is particularly true with the ten and fifteen per cent. strengths used.

3. *One Hour after Fertilization.*

The eggs of *Fundulus* cleave normally about two hours after fertilization. At one hour after fertilization, preparations must be well along in the cell for the formation of the first cleavage figures. The eggs seem considerably more resistant at this period as reference to the accompanying data will indicate (see Table IV.). This is no doubt due in part to the altered character

TABLE IV.

RESULTS OF TREATMENT OF EGGS WITH SEA-WATER SOLUTIONS OF ALCOHOL FOR ONE-HALF AND ONE HOUR AT ONE HOUR AFTER FERTILIZATION.

Series I. (treated for one-half hour).

Percentage of Alcohol.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing	Number Developing at End of Six Days.	Total Number Subnormal.	Percentage Subnormal.
Control	93	74	79.6	70	6	8.1
2	91	69	75.8	69	7	11.5
5	69	51	73.9	50	1	1.9
10	72	61	84.7	61	0	0
15	69	51	73.9	51	5	9.8
25	103	60	58.2	60	6	10 [1]

Series II. (treated for one hour).

Control (same as Series I.).						
2	74	59	79.6	57	6	10
5	80	60	75	58	10	16.7
10	73	57	78	56	2	3.5
15	100	60	60	58	8	13.3
25	85	6	7	6	4	66.7

[1] Most of the individuals of this lot were considerably weakened as compared with the control but not sufficiently to be classed as defective.

of the egg membranes. Also the nuclear material must be more deeply imbedded in the cytoplasm than in an earlier period, and thus less in position to be affected.

The treatment of the lot of eggs used at this time show that even the ten and fifteen per cent. solutions acting for a half hour do not produce a much greater effect than do the two per cent. and five per cent. solutions at an earlier period after fertilization. In the treatments for one hour, the fifteen per cent. and twenty-five per cent. solutions show a considerable effect in reducing the percentage developing; particularly is this true in the case of the latter concentration, which killed all of the eggs except six. Even with this rigorous treatment two individuals of the six went through to the sixth day as normal individuals and from all appearances would have hatched in about the normal time.

While the data at hand are not as extensive as perhaps might be desired, it seems safe to draw the conclusion that the eggs of *Fundulus* are very susceptible to toxic effects shortly after fertilization and become more resistant as development proceeds.

4. *Effects of Dilute Sodium Hydroxide Solutions.*

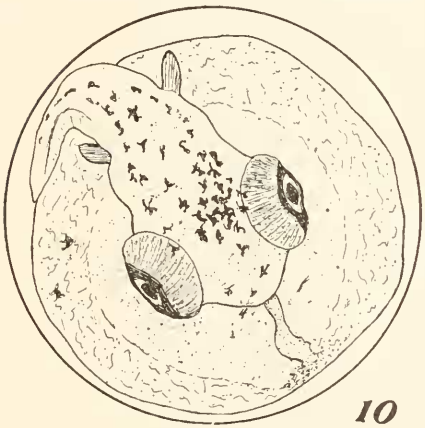
In several instances eggs were treated with dilutions of a standard alkali solution. The results show in some instances a large percentage of defective individuals from concentrations as low as $N/400$, $N/500$, and $N/800$ NaOH. The percentage of fertilizations is considerably lowered as the result of such treatments, and many aberrant cleavages occur. The effects in this connection, however, seem very variable and the time was not available for more than a few experiments, the data from which permit of no decided conclusions further than that some eggs are markedly affected in their development by treatments of low concentrations of alkali solutions. During last summer a lot of eggs treated with several drops of a very dilute solution of sodium hydroxide (0.6 c.c. $N/10$ NaOH + 50 c.c. sea water) for only fifteen minutes at twenty-five minutes after fertilization produced a large percentage of striking defects. One of the individuals of this lot is figured in the text (Fig. 14). The evidence is sufficient to state that the eggs of *Fundulus* in some instances in the more critical periods in their history before and after

fertilization may be much upset in their development by these alkali solutions of relatively low concentrations.

5. *Types of Defects.*

It is interesting and significant to note that treatments of the eggs for only fifteen and twenty minutes prior to fertilization produce most of the defective types secured by treatments for long periods, twenty-four hours or more, beginning in the early cleavage stages. Stockard ('10) has so thoroughly described these alcoholic types that it is only necessary here to refer to his paper on the effect of alcohol on the development of *Fundulus*.

Some of the type monsters secured are figured in the text. Several anophthalmic monsters were produced, one of those



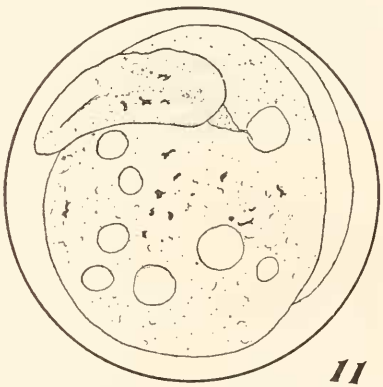


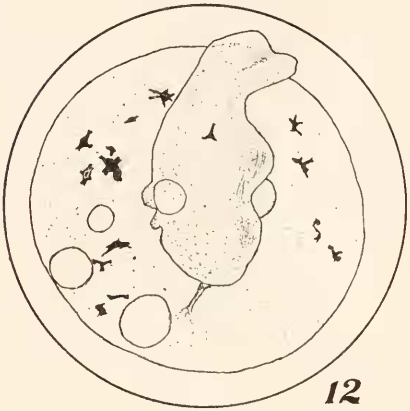
FIG. 10. A normal embryo six days old drawn to same scale as monsters.

FIG. 11. An anophthalmic monster of four days from egg treated with two per cent. alcohol in sea water prior to fertilization with untreated sperm.

figured showing a condition of spina bifida at the posterior end (Fig. 12). One or two cases of asymmetricum monophthalmicum (Fig. 13) occurred. Some showed the eyes curiously turned under towards each other on the ventral surface. Very few cases of cyclopia occurred, and these in eggs that showed an unusually large number of defective individuals, and it would be inferred that were in a very critical period at the time of treatment.

Quite a large proportion were generally defective, with much

shortened bodies, small eyes, a poor development of pigment, and a feeble circulation. A great assortment of defective individuals was produced in the lot of eggs treated with fifteen per cent. alcohol twenty minutes after fertilization, representing almost all of the types discussed by Stockard ('10). One of the microphthalmic monsters so developed is figured in the text (Fig. 17).



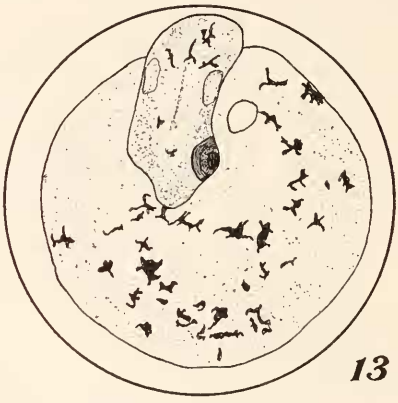


FIG. 12. An embryo six days old treated with two per cent. alcohol in sea water for fifteen minutes prior to fertilization with untreated sperm. A spina bifida, anophthalmic, with scarcely any pigmentation, and with a defective circulation.

FIG. 13. An asymmetricum monophthalmicum monster six days old from egg treated with two per cent. alcohol for fifteen minutes prior to fertilization with untreated sperm.

The term defective has come to signify to the mind a monster of an extreme type. So, in the tables and in many of the references in this paper the word sub-normal has been substituted. This is necessary in order to convey accurately the idea desired; for many of the individuals of the treated lot are those of much lowered vigor without any further special defect. Many embryos would not reach the six-day stage, but would disintegrate, the eggs becoming cloudy before that time. In a few instances after reaching about a late blastula or gastrula stage the embryo would slowly disintegrate. Thus it is to be noted that all grades of defective individuals occurred from aberrant cleavages to marked monsters of specific types and others which were merely slow in their rate of development and small in size.

IV. Treatment of Male Germ Cells.

1. *Length of Life of the Sperm of Fundulus.*

As has already been stated the length of life of the sperm of *Fundulus* after being stripped into sea water is surprisingly short. Newman ('06) has recorded that there is a definite spawning act in the breeding behavior; and in the light of this fact, the adaptiveness of this brief span of life is more readily understood. In this spawning act the copulating pair are in such position

Fig. 14. An embryo eleven days old which was treated twenty-five minutes after fertilization for fifteen minutes with dilute alkali solution (0.6 c.c. $N/10$ NaOH plus 50 c.c. sea water). A cyclopean monster, embryo twisted and short, with heart beat, and no circulation.

Fig. 15. A sixteen-day embryo developing from egg which was fertilized by sperm treated with fifteen per cent. alcohol in distilled water for three minutes, and afterwards activated with a weak alkali solution (0.6 c.c. $N/10$ NaOH plus 50 c.c. sea water). Microphthalmic monster, deformed in shape, yolk sac imperfectly absorbed, heart beat with no circulation.

that immediately upon the extrusion of the eggs the synchronous rhythmic discharge of the milt from the male occurs, and this places the spermatozoa in direct contact with the eggs to be fertilized.

It was necessary at the outset of a series of experiments of the character herein discussed to study the length of life of the sperm in various solutions. From the accompanying table (see Table V.) it will be noted that the duration of activity in a small drop —one tenth of a cubic centimeter—of distilled water is about one

minute or less. This factor held constant in the alcohol solutions in distilled water to as high as twenty per cent. In the earlier part of the experiments of the season of 1915, the spermatozoa were treated with the following strengths of alcohol in distilled water: 0.1 per cent., 0.2 per cent., 0.5 per cent., 1 per cent., 1.5 per cent., 2 per cent., 2.5 per cent., 2.8 per cent., 3 per cent., 3.5 per cent. and 4 per cent. At the end of a minute, in most instances, the spermatozoa were inert and consequently incapable of fertilizing an egg. It was found possible to activate them,

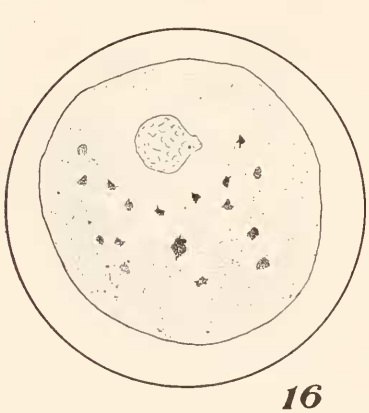


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FIG. 16. An embryo from same lot as the one in preceding figure with only remains of embryo disintegrating over yolk mass.

FIG. 17. A microphthalmic monster of six days treated with fifteen per cent. alcohol in sea water for fifteen minutes at twenty minutes after fertilization.

however, by the use of a weak sodium hydroxide solution (0.6 c.c. $N/10$ NaOH + 50 c.c. sea water, or better, a somewhat stronger solution 6 drops $N/10$ NaOH + 10 c.c. sea water). Just a drop of such a solution set the spermatozoa active in a short time, and in the treatments with the lower concentrations of alcohol, they were enabled to fertilize the eggs with a considerable percentage developing.

A large proportion of the spermatozoa could be activated in the treatments of lower concentrations and continue active for several minutes. It became evident therefore that best results were likely to be obtained by treatments of fifteen and twenty per cent. solutions. It was found possible to treat the spermatozoa

with twenty per cent. alcohol for even three minutes and activate a sufficient number to get some eggs fertilized. The small proportion which were revived to activity in this way indicated that most of the spermatozoa had been injured to a sufficient degree to cause their death.

Sea water solutions of alcohol produced very different results. The length of life was much longer in these, but except in rare instances, not a single spermatozoön after having ceased movement could be activated with the addition of the alkali solution. The resistance of the spermatozoa from a number of fish to the action of several solutions is given in the accompanying table (see Table V.). It will be observed that only in the highest percen-

TABLE V.

LENGTH OF LIFE OF SPERM OF *Fundulus* IN ONE TENTH OF A CUBIC CENTIMETER OF DISTILLED WATER, SEA WATER, AND GRADES OF ALCOHOL IN SEA WATER.[1]

Distilled Water.	Sea Water.	1 Per Cent. Alcohol.	2 Per Cent. Alcohol.	5 Per Cent. Alcohol.
1 min.	10 mins.	12 mins.	9 mins. 30 secs.	9 mins. 30 secs.
1 min. 10 secs.	8 mins.	8 mins.	8 mins.	9 mins.
45 secs.	14 mins.	10 mins.	8 mins. 30 secs.	9 mins.

10 Per Cent. Alcohol.	15 Per Cent. Alcohol.	20 Per Cent. Alcohol.	25 Per Cent. Alcohol.
8 mins.	5 mins. 30 secs.	2 mins.	killed at once.
9 mins.	8 mins. 30 secs.	3 mins.	30 secs.
7 mins. 30 secs.	7 mins.	2 mins. 30 secs.	killed at once.

tages is the time element satisfactorily eliminated. In these the greatest length of life is less than the shortest length found in the sea water alone. On this account, it was with these higher percentages that much of the work on the male germ cells was done.

The factor of dilution is another important one in the length of life of the sperm. A mass of spermatozoa placed in a large quantity of pure sea water became inactive in a very short time. In order to control the effect of dilution, the amount of solution used in these experiments was carefully measured and constant.

The causes of the inactivity of the sperm in distilled water,

[1] These periods represent the interval between the stripping of the milt from the male and the cessation of movement on the part of every spermatozoön in the dish. Consequently they represent the length of life of the most vigorous sperm in each lot.

and the striking behavior in activation with a dilute alkali afford a rather interesting problem. It is one of considerable complexity, related as it is so intimately with the mechanics of motility in the spermatozoön. The osmotic differences due to the behavior of different types of electrolytes is involved, and this is rather aside from the questions aimed at in this paper.

The main difficulty confronting one in the injury of the spermatozoa is to get such a dose and period of exposure as to cripple the spermatozöon and at the same time not deprive it of its motility and power of fertilization. Radium seems to do this to an excellent degree in some forms, but the matter is a much more difficult one with solutions of alcohol.

2. *Experiments on Treatment of Spermatozoa.*

In considering the experiments with the spermatozoön the problem of the differentiation of the effects of the alcohol on the spermatozoön and on the egg must be kept in mind. It was impossible to separate the treated spermatozoa from the treating agent and have them retain their fertilizing capacity. Yet as has been shown in the first part of this paper, low concentrations of alcohol, acting for short periods before and after fertilization, materially affect the developing eggs. Several factors served to reduce this effect, and on a comparative basis it would seem to eliminate it by establishing a differential.

It was found that five minutes' exposure of a control to the action of the sperm served to insure about as good a fertilization result as when allowed to stand for fifteen minutes. So with the exception of the experiment with the twenty per cent. solution of alcohol in distilled water, all of the eggs fertilized by treated spermatozoa, as well as the controls, were given five minutes for fertilization. The object of this was to reduce to a minimum the length of time that the alcohol acted on the eggs.

Then, again, over a hundred eggs carrying their surrounding fluid and some sea water from the bodies of the fish during the act of stripping served to bring to a very dilute percentage the one tenth of a cubic centimeter of alcohol used. In the case of the treated mass of sperm cells, the alcohol was diluted not only by the milt of the male, but also by the fluid of the eggs added.

Two controls were operated in each case. One of these consisted in normal eggs fertilized with untreated spermatozoa. In the other the eggs were treated with an equal amount of alcohol to that used in the other dishes of the experiment. They were then washed and fertilized with untreated spermatozoa. Such treatment as this should, for reasons mentioned above, quite fully meet the requirements of a control.

An attempt was made to alcoholize the males. Several of these were placed in various concentrations of alcohol, but the percentage in which they will live for any length of time is so dilute that this method was soon abandoned. The difficulties of acclimatization and of proper aëration seem to render this sort of experiment an impractical one.

(*a*) *Effects of Twenty Per Cent. Alcohol in Distilled Water.*— The spermatozoa treated with two tenths of a cubic centimeter of this strength of alcohol became motionless in less than a minute. They were allowed to remain in the solution for two and three minutes. Then they were activated with a dilute solution of sodium hydroxide. The lot of eggs were rather weak to start with, as is indicated by the twenty per cent. sub-normal which occurred in the control (see Table VI.). However, the number

TABLE VI.

EFFECTS OF TWENTY PER CENT. DISTILLED WATER SOLUTION OF ALCOHOL ON THE SPERM OF *Fundulus*.

Treatment	Total Number of Eggs in Lot	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number of Aberrant Cleavages Subsequently Disintegrating.	Number Developing at End of Sixth Day.	Total Number Sub-normal.	Percentage Sub-normal.
Control	103	77	74.7	0	64	16	20.7
Eggs treated with 0.2 c.c. of 20 per cent. alc. + 0.2 c.c. alkali[1] for 15 minutes	106	13	12.2	3	6	7	53.7
Spermatozoa treated with 0.2 c.c. of 20 per cent. alcohol for 3 minutes and activated with 0.2 c.c. alkali	152	0	0	9	0	0	—
Same treatment of sperm except for two minutes	128	8	6.2	9	5	3	37.5
Same treatment of sperm for two minutes	116	7	6	5	3	7	100

[1] The strength alkali used for activating was six drops of $N/10$ NaOH + 10 c.c. sea water.

TABLE VII.

SHOWING EFFECTS OF FIFTEEN PER CENT. ALCOHOL IN SEA WATER SOLUTION UPON THE SPERM OF *Fundulus*.

Treatment.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Sixth Day.	Total Number Subnormal.	Percentage Subnormal.
Series I.						
Control	61	25	41	24	1	4
Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol	77	15	19.4	15	0	0
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 5 minutes	145	77	53.1	72	5	6.5
Series II.						
Control	163	62	38	58	4	6.5
Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol	119	20	16.8	13	7	35
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute	194	22	11.3	16	6	27.3
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 5 minutes	149	5	3.4	4	2	40
Series III.						
Control	57	28	49.1	28	0	0
Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol	65	31	47.7	31	0	0
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute	62	42	67.7	42	3	7.1
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 6 minutes	94	26	27.6	26	1	3.8
Series IV.						
Control	62	27	43.5	25	3	11.1
Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol	61	9	14.7	9	1	11.1
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute	102	25	24.5	21	6	24
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 7 minutes	121	3	2.5	3	0	0

of eggs represented and the fact that they were all from the same lot seems to eliminate this objection.

Where the spermatozoa were treated for three minutes, none of the eggs fertilized developed beyond the irregular early cleavages. There were nine of these, all of which subsequently disintegrated. Of the eggs fertilized with those treated for two minutes, one lot had five developing at the end of six days, all of which were normal. However, three of the eight which passed the early cleavage stages had disintegrated by the fifth day; and there

were nine aberrant cleavages which early disintegrated. Another lot similarly treated showed even more decided effects; for of the seven developing beyond the early cleavages, all were defective at the end of the sixth day.

These results seem to warrant the conclusion that a treatment of this strength is injurious to the sperm as well as to the egg. This is most clearly indicated in the instance where the spermatozoa were treated for three minutes.

(*b*) *Effects of Fifteen Per Cent. Alcohol in Sea Water.*—The effects of this strength solution are not so marked either on the eggs or on the sperm as was the solution used in the experiments just discussed. In two instances there were no sub-normal individuals developing from the lot of eggs treated for five minutes prior to fertilization, though in most of the dishes of eggs so treated, the percentage developing was considerably below that of the control. The percentage sub-normal in Series I. (Table VII.) as the result of treating the sperm for five minutes is not very appreciably different from that in the control.

There is, however, a larger percentage developing in this dish; a fact which indicates a greater vigor on the part of the spermatozoa of this male, as well as perhaps a stimulating effect on the part of the alcohol. This is also shown by the accelerated cleavage on the part of the eggs in this dish, the early cleavages having occurred several minutes before those in the other dishes of the series.

In the second series (Table VII.) the treatment of the eggs prior to fertilization shows a decided effect, thirty-five per cent. developing sub-normally. The treatment of the sperm for one minute seems in this series to have less effect than the treatment of the eggs alone prior to fertilization. Out of the five developing beyond the early cleavages from the fertilizations with the spermatozoa treated for five minutes, two resulted in defective embryos.

In the other two series with this strength, the longer treatment seems to have a selective action on the sperm, a much smaller percentage of sub-normals occurring than in the one minute treatments. The strong spermatozoa seem to have been selected by resisting the treatment, and while the percentage developing

in these cases is much lowered, those that do develop seem to be more vigorous. This is indicated by the large percentage developing to the sixth day as normal individuals.

In the summarized results of these four experiments (see Table VIII.), it appears that the treatments of one minute

TABLE VIII.

SUMMARY OF RESULTS OF FIFTEEN PER CENT. ALCOHOL IN SEA WATER ON THE SPERM OF *Fundulus*.

Treatment.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Sixth Day.	Total Number Sub-normal.	Percentage Sub-normal.
Control	343	142	41.4	135	8	5.6
Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol	322	75	23.3	68	8	10.6
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute	358	89	24.8	79	15	16.8
Sperm treated with 0.1 c.c. 15 per cent. alcohol for five or more minutes	509	111	21.8	105	8	7.2

duration have a greater effect than does the longer treatment. The shorter treatment seems to cripple the weaker spermatozoa, which fertilize the eggs and result in sub-normal individuals; while the longer treatment weeds out the weaker ones, the strongest ones being unaffected and leading to normal development when they fertilize the egg.

(*c*) *Comparative Effects of Treatments of Different Lengths.*—One method used in determining whether the treatment was effective on the spermatozoa was to give them equal doses for different lengths of time. In such an experiment the effect on the eggs should be a constant factor, and a differential thereby established between the injury of the spermatozoa treated for one minute and those treated for five minutes or longer. Five such series were carried out and the combined results are given in tabulated form (see Table IX.).

Examination of these data will indicate that while the percentage sub-normal is not very large in any instance, the greater effect is with the one minute treatment. The longer treatments

TABLE IX.

COMBINED RESULTS OF FIVE SERIES OF EXPERIMENTS SHOWING DIFFERENCES IN EFFECTS OF ONE MINUTE AND FIVE OR MORE MINUTES' TREATMENT OF THE SPERM OF *Fundulus* WITH FIFTEEN PER CENT. ALCOHOL.

Treatment.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Sixth Day.	Total Number Subnormal.	Percentage Subnormal.
Control	495	213	43.3	206	9	4.2
Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute	607	204	33.6	191	31	15.1
Sperm treated with 0.1 c.c. 15 per cent. alcohol for five or more minutes	648	43	6.6	42	3	7

lead to the elimination of the weaker lot, and while the percentage developing is much smaller, subnormal individuals are rarer than in the control.

(*d*) *Selective Action with Treatment of Ten Per Cent. Alcohol.*— Three separate lots of spermatozoa were treated with two tenths of a cubic centimeter of ten per cent. alcohol in sea water for such a length of time that only a few were left active in the dish. A lot of eggs were then mixed with the treated drop of milt and allowed to stand five minutes for fertilization. In one instance

TABLE X.

SHOWING THE EFFECTS OF TEN PER CENT. ALCOHOL IN SEA WATER ON THE SPERM OF *Fundulus*.

Treatment.	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Sixth Day.	Total Number Subnormal.	Percentage Subnormal.
Control	127	81	63.7	81	1	1.2
Eggs treated for five minutes prior to fertilization with 0.2 c.c. of 10 per cent. alcohol	144	67	46.5	67	5	7.5
Sperm treated with 0.2 c.c. 10 per cent. alcohol for such a length of time as only a few were left active.	200	2	1	2	0	0
Sperm treated with 0.2 c.c. 10 per cent. alcohol for such a length of time as only a few were left active.	195	2	1	2	0	0
Sperm treated with 0.2 c.c. 10 per cent. alcohol for such a length of time as only a few were left active.	203	0	0	0	0	—

no fertilizations were secured; in the others, only two and these were developing normally at the end of the sixth day.

There seems to be very plainly exhibited in this experiment an eliminating action of the alcohol on the spermatozoa. The treatment was of sufficient length to kill out all of the weaker ones, and the stronger ones fertilized the eggs with resulting normal individuals.

(*e*) *Effects of Methylene Blue Solution on Eggs Prior to Fertilization.*—As stated earlier in this paper, the results of the Hertwigs ('13) on the treatment of the sperm with methylene blue seem somewhat contradictory in nature. Treatment of the spermatozoa of *Gobius jozo* for one hour in 0.02 per cent. and 0.1 per cent. solutions showed a decidedly injurious effect when used to fertilize the eggs of the same species of fish. When the spermatozoa were treated for forty-five minutes with the 0.1 per cent. solution and used to fertilize the eggs of another fish, *Crenilabrus pavo*, the resulting development showed no effect of this treatment.

In the light of the experiments reported in the first section of this paper, it appeared that the discrepancy might be explained as due to the difference in effect upon the eggs of the two fishes of the methylene blue in the sperm suspension used to fertilize the eggs of the fish.

TABLE XI.

RESULTS OF TREATING EGGS OF *Fundulus heteroclitus* WITH SOLUTIONS OF METHYLENE BLUE FOR FIFTEEN MINUTES PRIOR TO FERTILIZATION.

Percentage of Methylene Blue	Total Number of Eggs in Lot.	Number Developing Beyond Early Cleavages.	Percentage Developing.	Number Developing at End of Sixth Day.	Total Number Sub-normal.	Percentage Sub-normal.
Control	130	24	17	24	0	0
0.02 per cent. methylene blue in sea water	99	16	16.1	16	8	50
0.1 per cent. methylene blue in sea water	104	17	16.3	14	7	41.1
0.02 per cent. methylene blue in distilled water	131	46	35.1	46	5	10.8
0.1 per cent. methylene blue in distilled water	104	11	10.6	9	3	27.2

Accordingly an experiment was planned to test the plausibility of this view when applied to the eggs of *Fundulus heteroclitus*. The results of this experiment are given in an accompanying table (see Table XI.). While the matter was not exhaustively

followed out, the high percentage of sub-normals resulting seems to suggest that this is a factor that should at least be carefully controlled, and the effects they have reported as being due to the toxic action of the methylene blue on the sperm may very probably have been due in considerable part to the action of the methylene blue added with the sperm upon the eggs themselves.

While only a low percentage of the control eggs developed in this experiment—on account of the delayed fertilization—a very striking abundance of subnormal individuals were produced in the methylene blue series and no such specimens were recorded in the control. This fact would seem to indicate in a positive way the effects of such solutions on the development of the eggs. The eggs of *Fundulus* are very probably much more hardy and resistant to all treatments than are those of *Gobius*, judging from the experiments reported in the literature on the two forms.

Discussion.

The results reported in this paper seem to lead to rather definite conclusions. One of these is that the period in the egg prior to fertilization is a critical one. Dosages of alcohol which are comparatively negligible in their action an hour after fertilization produce marked effects at this earlier period. Also the period very shortly after fertilization seems another time at which the egg is more susceptible to injury.

In analyzing results such as these one is impressed with the number of factors involved. Different eggs at the same period in their development differ in their resistance to the same substances in solution. A lethal dose for one is comparatively slight in its effect upon another. A part of this results no doubt from the differences in permeability of the individual eggs in the lot involved due to their slightly different metabolic or developmental states. As a consequence more of the toxic substance passes through the membranes of certain eggs to affect their protoplasmic content than through the membranes of others.

Just before fertilization, the maturation processes are in operation in the egg. Immediately after fertilization these are being completed, and the male and female pronuclei are near the surface of the protoplasmic disc of the egg. The effect of

the treatments with alcohol at these periods would certainly be due in part to its effect on the nuclear material. The nature of this effect one can little more than surmise. Alcohol has an affinity for water, and would tend to remove this from the protoplasm of the egg. On the other hand, it may act to alter to some degree the actual chemical composition of the nuclear material.

One is led naturally to the position that in the case of the eggs of other forms, perhaps even of mammals, similar critical periods may exist. If so, this finding is an important one; for it may be that sudden acute intoxication of the female parent about the time of conception may lead to the abnormality of the resulting embryo, or may even prevent the fertilization of the egg. There are numerous instances cited to support such a view in medical statistics. A somewhat different statement of the case is that acute intoxication of the eggs at critical periods in their history may act to prevent development altogether, or to render it abnormal in a considerable proportion of cases.

The spermatozoa show a surprising degree of resistance to the action of alcohol solutions in both sea water and distilled water. Treatments with the higher percentages of alcohol yielded the best results. Here one could be reasonably sure that one was injuring the spermatozoa; for they lived a much shorter time in these concentrations than they do in pure sea water.

While the results are not as clean cut in the experiments on the sperm as in the case of the egg, there does seem to be in several instances a definite injury to the sperm. In others the action of the alcohol seems a selective one. Still the results here are much complicated by the action of the treating reagent on the egg prior and just subsequent to fertilization, even though the greatest care was taken to closely control this factor.

Summary.

1. The eggs of *Fundulus heteroclitus* are very susceptible to injury from treatment with low concentrations of alcohol prior to fertilization. This period seems an especially critical one in the history of the egg.

2. Two per cent. and five per cent. solutions of alcohol in sea

water acting for short lengths of time prior to fertilization reduce to a marked degree the number of eggs which develop.

3. Such treatment of the eggs of *Fundulus* produce a number of aberrant cleavages. These seem to be due in part to an effect upon the chromatin of the egg.

4. A large proportion of the individuals which develop from eggs treated with alcohol prior to fertilization are markedly defective.

5. Twenty minutes after fertilization the eggs are much more sensitive to injury with alcohol than at one hour after fertilization and in the early cleavages.

6. The types of defects produced by these acute treatments were of all grades from aberrant cleavages to marked monsters of specific types, and others which were merely slow in their rate of development and small in size.

7. Dilute solutions of sodium hydroxide acting prior to fertilization produced in some eggs much the same effects as secured from treatments with alcohol.

8. The spermatozoa of *Fundulus* usually continue active for less than a minute after being stripped in one tenth of a cubic centimeter of distilled water, and in solutions of alcohol in distilled water. After complete cessation of movement in these solutions, some of the spermatozoa may be activated with a dilute sodium hydroxide solution sufficiently to fertilize an egg.

9. In one tenth of a cubic centimeter of sea water and in the weaker concentrations of alcohol in sea water, the spermatozoa live for several minutes after being stripped. After cessation of movement in these solutions the spermatozoa very rarely activate upon the addition of an alkali solution.

10. The higher concentrations of alcohol acting for short periods on the spermatozoa seem to injure many of them without depriving them of their fertilizing power. When acting for longer periods, these same concentrations, in some instances, clearly eliminate the weaker spermatozoa and the resistant ones which survive are often capable of producing normal fertilization and development.

BIBLIOGRAPHY.

Dungay, N. S.

'13 A Study of the Effects of Injury Upon the Fertilizing Power of Sperm. Biol. Bu.l., Vol 24.

Hertwig, G.

'11 Radiumbestrahlung unbefruchtete Froscheier und ihre Entwicklung nach Befruchtung mit normalen Samen. Archiv. f. Mikr. Anat., Bd. 77, Abt. II.

'12 Das Schicksal des mit Radium bestrahlten Spermachromatins in Seeigelei. Archiv. f. Mikr. Anat., Bd. 79, Abt. II.

'13 Parthenogenesis bei Wirbeltieren, hervorgerufen durch artfremden radiumbestrahlten Samen. Archiv. f. Mikr. Anat., Bd. 81, Abt. II.

Hertwig, G., and Paula.

'13 Beeinflussung der männlichen Keimzellen durch chemische Stoffe. Archiv. F. Mikr. Anat., Bd. 83, Abt. II.

Hertwig, O.

'11 Die Radiumkrankheit tierischer Keimzellen. Bonn. Fr. Cohen.

Loeb, J.

'13 Artificial parthenogenesis and fertilization. Univ. of Chicago Press.

Newman, H. H.

'06 Spawning Behavior and Sexual Dimorphism in Fundulus heteroclitus and Allied Fish. BIOL. BULL., Vol. 12.

Oppermann, K.

'13 Die Entwicklung von Forelleneiern nach Befruchtung mit radiumbestrahlten Samenfäden. Archiv. f. Mikr. Anat., Bd. 83, Abt. II.

Stockard, C. R.

'10 The Influence of Alcohol and Other Anæsthetics on Embryonic Development. Amer. Journ. Anat., Vol. 10.

'11 An Experimental Study of the Influence of Alcohol on the Germ Cells and the Developing Embryos of Mammals. Archiv. f. Entw. Mech., Bd. 35.

'13 The Effect on the Offspring of Intoxicating the Male Parent and the Transmission of the Defects to Subsequent Generations. Amer. Nat., Vol. 47.

'16 A Further Analysis of the Hereditary Transmission of Degeneracy and Deformities by the Descendants of Alcoholized Mammals. Amer. Nat., Vol. 50.

THE EYE OF THE PARASITIC COPEPOD, SALMINCOLA EDWARDSII OLSSON (LERNÆOPODA EDWARDSII OLSSON).

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Contents.

Introductory Remarks.

The material from which the following studies were made consisted of numerous free-living larvæ of *Salmincola edwardsii* (*Lernæopoda edwardsii*) Olsson, a parasitic copepod of the family Lernæopodidæ, which infests the common brook-trout, *Salvelinus fontinalis*. In three former papers (Fasten '12, '13, '14) the author has discussed the economic importance, the behavior and the fertilization process of the parasite. In this publication, the structure of the eye will be described.

Wilson (1911) in his paper on the development of *Achtheres ambloplitis* Kellicott, one of the Lernæopodidæ observes that the eye is rudimentary in character and is only developed during the metanauplius stage, while the organism is still surrounded by its embryonic membranes. Wilson says, "the extremely rudimentary eye (*e*) can now be distinguished inside the coils of the attachment filament. It is made up of three ovate ocelli, two dorso-lateral and one inferomedian, which are entirely separated from one another and devoid of pigment. The structure of each ocellus has also degenerated until all that remains is a more or less granular mass, staining deeply in hæmatoxylin and containing near its anterior end three lighter spots. No trace of lenses can be found in any of the sections and the entire

structure disappears during the next stage." In a later paper (Wilson, '15), on the Lernæopodidæ, this same author states the following: "The eye in this whole family is extremely rudimentary, appears only for a short time during the development stages, and then entirely disappears." In *Salmincola edwardsii*, which also belongs to this family of Lernæopodidæ, the eye is well developed and resembles to a marked degree the visual organ of the free-living marine copepod *Eucalanus elongatus* Dana, worked on by Esterly ('08). Furthermore, during the metanauplius stage, the eye of *Salmincola edwardsii* makes its appearance and attains its full development in the free-swimming larval form, the so-called first copepodid stage.

Methods.

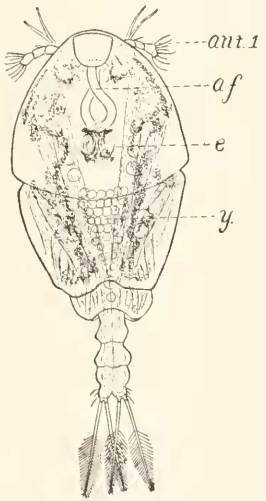
Entire mounts as well as sections of the larvæ were used for this study. Various fluids, such as Bouin's, Gilson's and 5 per cent. corrosive-acetic were tried for fixation, but the last-mentioned reagent yielded the best results and was used almost exclusively.

The entire mounts were made in the following manner. The organisms were placed in 5 per cent. corrosive-acetic fluid for ten minutes or longer, and then they were washed in many changes of water. After this they were run up through the various grades of alcohol, being left about ten minutes in each, and finally they were cleared in xylol and mounted in balsam. Larvæ thus treated yielded beautiful results, showing little change from the normal condition. Those which were allowed to remain in the fixative longer than ten minutes, generally had most of their pigments dissolved out, thereby making it possible to obtain a fine view of the external structure of the eye.

The larvæ to be sectioned were also fixed in the corrosive-acetic mixture. After dehydrating, clearing and infiltrating, the organisms were permanently imbedded in paraffine for sectioning. The sections were cut from 3–6 μ in thickness, in frontal, transverse and sagittal planes, and were stained in Heidenhain's iron-hæmatoxylin, with a counterstain of acid-fuchsin or eosin. These sections were very helpful in determining the internal structure of the eye.

Gross Structure of the Eye.

The eye of *Salmincola edwardsii* is located in the cephalothorax, occupying a central position, directly below the loop of the attachment filament. Text figure *A*, which is a dorsal view of the larval free-swimming stage, shows the location of the eye (*e*). When viewed from the dorsal or the ventral surface of the free-swimming larva, the eye appears as a more or less x-shaped, reddish-brown pigment blotch in which three ocelli



Text Figure *A*. Dorsal view of free-swimming larva of *Salmincola edwardsii* (*Lernæopoda edwardsii*), showing the position of the eye. × 86.8 *a.f.* = attachment filament. *ant.*1 = first antennæ. *e.* = tripartite eye. *y.* = yolk.

can be distinguished. Two of these are situated dorso-laterally, while the third is placed immediately beneath them, occupying a median position. This is shown in Figs. 1 and 2, which are enlarged drawings of the eye, as seen respectively from the ventral and dorsal sides of the animal.

When favorable preparations of the eye, from which the pigment has been extracted, are studied under the high power objectives, the external structure of the ocelli becomes more apparent. In such preparations, each ocellus is seen to be embedded in a semi-lunar cup (Figs. 1 and 2, *c*), and is covered by a cuticular outer surface, which is divided up into narrow bands by means of transverse striations. These bands are

further crossed by vertical lines breaking them up into small squares. The surface of each ocellus thus appears to be made up of numerous facets, very similar to the facets of ommatidia. Figs. 1 and 2 show this appearance. Fig. 1, which is a drawing of a ventral view of the eye, shows the facet-like surfaces and the semi-lunar cups particularly well.

Internal Structure of the Eye.

The true structure of the eye is revealed when sections of the organ are studied under the microscope. In Fig. 3, which is a transverse section of the larval organism, the tripartite eye (*e*) is seen to occupy the middle space between the brain (*b*), and the dorsal wall of the body (*w*). The details of the eye can best be seen in Figs. 4 and 5. Fig. 4 is an enlarged camera-lucida drawing of the eye seen in Fig. 3, while Fig. 5 is a drawing of a frontal section of the eye. In size, the two lateral ocelli (Fig. 4, *l. o*) are equal, while the median ocellus (Fig. 4, *m. o*) measures about two thirds the dimensions of either of the aforementioned ones. Furthermore, as already stated, these ocelli are imbedded in semi-lunar cups (Figs. 4 and 5, *c*) which touch each other closely. The inner surface of each cup is thickened into a basal plate (Figs. 4 and 5, *r*) which stains a heavy black with Heidenhain's iron-hæmatoxylin. This plate comes in contact with the ocellus and, in all probability, is its most sensitive portion. Esterly ('08) found that in *Eucalanus elongatus* the lateral ocelli possessed two basal plates, while the median ocellus contained only one. In *Salmincola edwardsii* this difference was not observed. Here each ocellus bears a single plate. Between the open spaces of the semi-lunar cups the pigment granules of the eye are found distributed (Fig. 4, *p*).

Upon closer examination each ocellus is observed to consist of a definite number of cells, the so-called retinal cells (see Figs. 4 and 5), there being nine in either of the lateral ocelli, and five in the median one. This was determined by careful reconstructions of transverse, frontal and sagittal sections of the visual organ. In *Eucalanus elongatus*, Esterly found that the lateral ocelli also contained nine retinal cells, but that the median one possessed ten of them.

Within every retinal cell, there is a prominent nucleus, more or less spherical in appearance (Figs. 4 and 5, *n*), which is made up of a network, consisting of fine chromatic strands with thickened clumps of chromatin. At the base of the cell, that portion nearest the basal plate of the ocellus, there is a rod-like, heavily staining structure surrounded by a clear space. This is the phaosome (Figs. 4 and 5, *f*), and in all probability it functions in the transmission of visual stimuli to the nerves of the retinal cells. Esterly found numbers of these bodies distributed randomly through the retinal cells of *Eucalanus elongatus*. In *Salmincola edwardsii*, however, this was not found to be the case. Here there is but one phaosome to each retinal cell and this occupies a definite position between the nucleus and the basal plate of the ocellus. No definite lenses are present in the ocelli of *Salmincola edwardsii*.

The nerves of the retinal cells make their way posteriorly, from the surfaces of the semi-lunar cups. These nerves are very thin, fine strands which cannot be counted with even the highest powers of the microscope. But assuming that each retinal cell is connected with one nerve, there must be nine retinal nerves to each lateral ocellus, and five of them to the median ocellus, making altogether twenty-three nerves. Slightly back of the ocelli, these nerves combine into an optic nerve (Fig. 5, *o. n*) and this then enters the brain of the larval organism.

Summary.

1. The eye of *Salmincola edwardsii* Olsson is located medianally, in the space between the brain and the dorsal wall of the body.

2. Unlike the eye of *Achtheres ambloplitis* Kellicott another one of the Lernæopodidæ, described by Wilson, the visual organ of *Salmincola edwardsii* is normally developed and functions during the first copepodid or the free-swimming larval stage of the parasitic organism.

3. The eye is more or less of a reddish-brown, x-shaped pigment blotch, consisting of three ocelli. Two ocelli are located laterally, while the third is below these, occupying a median position.

4. In size, the median ocellus is about two thirds the dimensions of either of the lateral ones.

5. Each ocellus is constructed somewhat similarly. It is embedded in a semi-lunar cup whose internal surface is thickened into a basal plate. Covering the external face of the ocellus is a cuticle which is divided up into squares that appear like the facets of ommatidia.

6. Interiorly every ocellus contains numerous retinal cells. There are nine of these cells in each lateral ocellus and five of them in the median ocellus.

7. The retinal cell possesses a large, rounded nucleus and a single rod-like, heavily staining phaosome, which is located between the nucleus and the basal plate.

8. The optic nerve which makes its way from the ocelli to the brain consists, in all probability, of twenty-three nerves corresponding to the number of retinal cells found in the eye.

BIBLIOGRAPHY.

Esterly, C. O.

'08 The Light Recipient Organs of the Copepod Eucalanus elongatus. Bul. Mus. Comp. Zool. Harvard College, Vol. 53, No. 1, pp. 3–56.

Fasten, N.

'12 The Brook Trout Disease at Wild Rose and Other Hatcheries. (The Brook Trout Disease in Wisconsin Waters.) Rep't Wis. Fish Com., 1911–1912, pp. 12–22.

'13 The Behavior of a Parasitic Copepod, Lernæopoda edwardsii Olsson. Jour. of An. Beh., Vol. 3, pp. 36–60.

'14 Fertilization in the Parasitic Copepod, Lernæopoda edwardsii Olsson. Biol. Bul., Vol. 27, No. 2, pp. 115–127.

Wilson, C. B.

'11 North American Parasitic Copepods. Part 9. The Lernaeopodidae. Proc. U. S. Nat. Mus., Vol. 39, pp. 189–226.

'15 North American Parasitic Copepods Belonging to the Lernaeopodidae, with a Revision of the Entire Family. Proc. U. S. Nat. Mus., Vol. 47, pp. 565-729.

EXPLANATION OF PLATES.

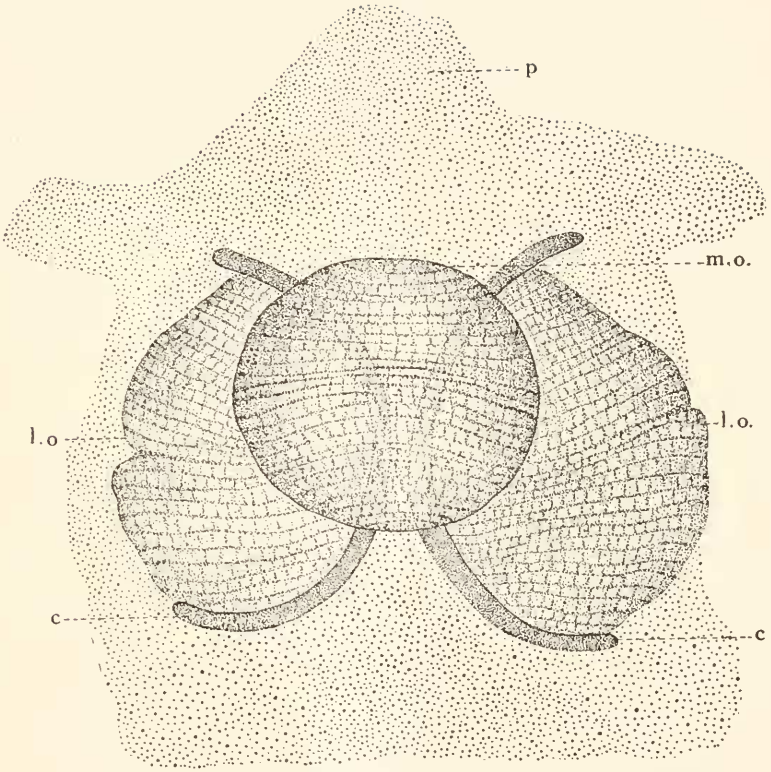
All drawings were made with the aid of the camera lucida. The magnification of each figure is given after its description.

Abbreviations.

b. = brain.
c. = semi-lunar cups of ocelli.
ch. = chitinous membrane of larva.
e. = tripartite eye.
f. = phaosomes.
l.o. = lateral ocellus.
m. = dorsal muscles.
m.o. = median ocellus.
mxp. g. = maxillipedal gland.
n. = nuclei of retinal cells.
oe. = oesophagus.
o.n. = optic nerve.
p. = pigment of eye.
r. = basal plates of ocelli.
w. = body wall.

Explanation of Plate I.

Fig. 1. View of the tripartite eye as seen from the ventral surface of the larval organism. The facet-like surfaces of the lateral (*l.o.*) and median (*m.o.*) ocelli as well as the semi-lunar cups (*c*), and the pigment (*p*), can readily be observed. × 1,637.



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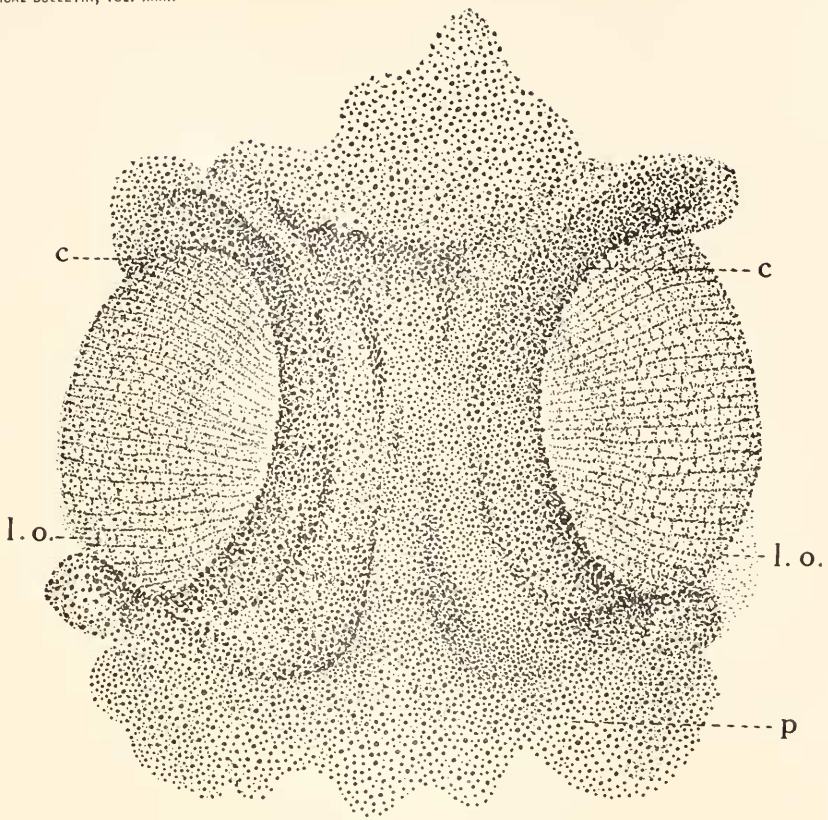
Explanation of Plate II.

Fig. 2. View of the eye as seen from the dorsal surface of the free-swimming larva. The distribution of the pigment (*p*) is here seen to good advantage. The facet-like surface of the ocelli may also be observed. × 1,760.

Fig. 3. Cross-section of a larval organism through the region of the eye. The position of the eye (*e*) is noticed to be between the brain (*b*) and the dorsal surface of the body wall (*w*). × 460.



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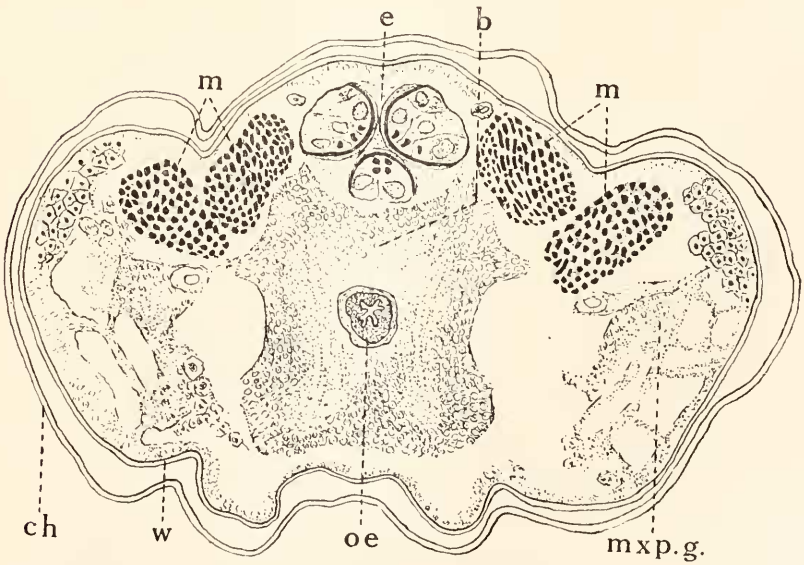

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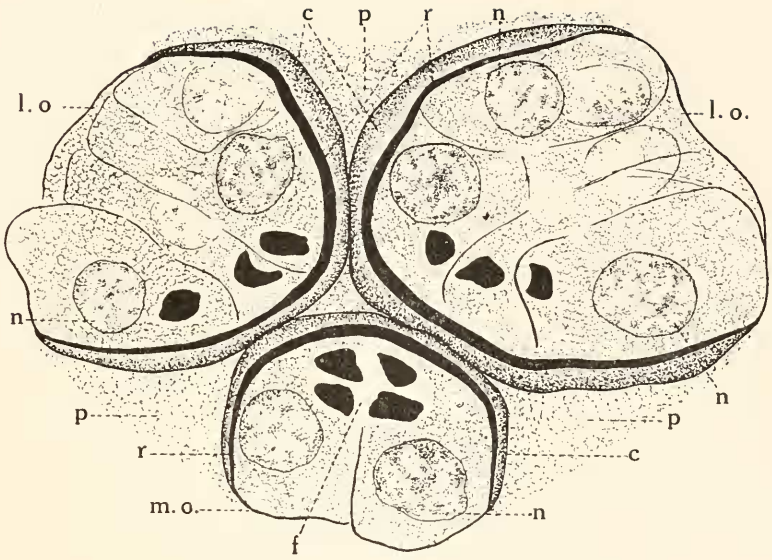
Explanation of Plate III.

Fig. 4. Enlarged drawing of the tripartite eye observed in Fig. 3, to show the details of structure. × 1,928.

Fig. 5. Frontal section of the eye, showing details of internal structure, and also the optic nerve (*o.n.*). × 1,272.



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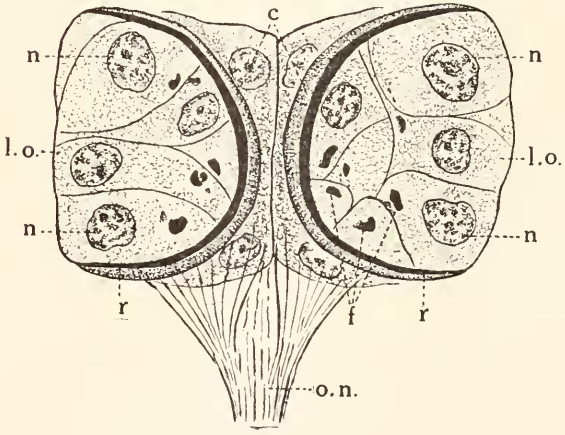

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FURTHER OBSERVATIONS ON AXIAL SUSCEPTIBILITY GRADIENTS IN ALGÆ.

C. M. CHILD.

With two Figures.

Introductory.

In a recent paper (Child, '16*a*) it was shown that axial gradients in susceptibility to cyanides and various other agents are characteristic features of some fourteen species of axiate marine algæ, and the question of the significance of these gradients in relation to polarity and developmental order was considered.

The present paper records observations made during the summer of 1916 at the Marine Biological Laboratory, Woods Hole. It is concerned primarily with the demonstration of what may be called the normal gradients in the species examined, *i. e.*, the gradients characteristic of the plant in good physiological condition under good or average rather than extreme, environmental conditions, but some of the alterations resulting from altered environment are briefly described. In addition to the fourteen species of the earlier paper, eleven more species have been examined, at least in part, with definite results in every case.

The method used is essentially the same that was employed to demonstrate the axial gradients in various other animal and plant species (Child, '13*b*, '14, '15*a*, '15*c*, Chap. III., '16*a*, '16*b*). It consists in determining the susceptibility to, *i. e.*, the survival time in, a certain concentration of an agent which kills within a few hours, but not immediately. The differences in susceptibility as determined by the differences in survival-time along an axis or in different organs are in general an indication of the differences in physiological condition. The relations between susceptibility to inhibiting agents and physiological, metabolic or protoplasmic condition have been discussed elsewhere (Child, '13*a*, '15*b*, Chap. III., '16*a*) and require no further consideration at present.

The time of death of the plant cells is approximately deter-

mined by the visible changes in aggregate condition of the protoplasm, and in many of the red and brown algæ by the diffusion of the pigment out of the chromatophores and out of the cell. The protoplasmic changes are in many cases much more readily seen if the plant has been previously stained with neutral red. The general character of the death changes has been described (Child, '16*a*) and some special observations are recorded below.

In the work of 1915 the chief agents used to measure susceptibility were KCN, ethyl alcohol and the so-called vital dye, neutral red. In 1916 various other reagents in addition to these three were used, including ethyl ether, HCl, $CuSO_4$ and $HgCl_2$.

The Susceptibility Gradients in the Thalli.

The chief result of this further study is the same as that of the earlier, viz., that in the definitely axiate forms or parts examined a gradient in susceptibility exists along the axis, the apical region being primarily most, the basal least susceptible to toxic agents in high concentration. The regularity of this gradient is most marked in plants in good physiological condition and in the younger axes or the younger portions of axes. In some forms the original gradient may persist throughout the length of the axis, at least during the vegetative period, while in others it may undergo modification in the later stages or in the older regions of the body. In general it is also true that the more definitely axiate and orderly the growth form of the plant, the more definite and regular the susceptibility relations between different parts.

Since different species behave somewhat differently and require different modifications of method the data for each form examined are briefly given. The genera include, among the Chlorophyceæ, only *Bryopsis* and *Cladophora*, among the Phaeophyceæ, *Fucus*, and among the Rhodophyceæ, *Chondrus*, *Cystoclonium*, *Agardhiella*, *Lomentaria*, *Griffithsia*, *Callithamnion*.

Bryopsis plumosa.

The demonstration of an axial gradient in this form seemed to me of particular interest since the whole plant body consisting of creeping rhizome-like axes from which arise vertical axes with

a highly orderly pinnate arrangement of lateral branches is a single cell. Unfortunately, owing to scarcity of material, it was possible to examine only a few of the vertical axes with their branches and these had been in standing water in the laboratory for twenty-four hours before they were available. They were first stained in neutral red and then placed in KCN *m*/50 in Syracuse dishes covered with a thin glass plate and the course of death observed under the microscope.

In those axes which were still in good condition death began in general at the apical end of each main axis and branch and progressed basipetally and in each system of main axis and branches as a whole a similar gradient appeared, the branches nearest the apical end being most susceptible and death progressing basipetally from branch to branch. Moreover, at least the younger branches were more susceptible than the level of the axes from which they arose.

It would, I think, be difficult to find a more beautiful example of intracellular axial gradients in susceptibility than in *Bryopsis*. As in other forms (Child, '16*a*) the first indication of approaching death is a deepening of the neutral red tint in the cell as if the protoplasm were becoming more acid. This change in color occurs first apically and progresses basipetally and is followed in a few moments by the disintegrative changes in the protoplast. The progress of the coagulation and aggregation of the protoplasm into masses which are at first almost black in consequence of the high concentration of the neutral red in them, but which lose the stain soon after coagulation, can be followed under the microscope from one level to another as a visible wave of change.

As stated above, the course of death is in general basipetal, but in the few axes examined there was none which did not show some irregularities. In young growing axes the irregularities are much less frequent than in old, where most or all of the branches have completed their growth, and a larger or smaller number of the more basal branches may be in part or entirely dead when the plant is collected. Similarly, the more apical younger portions of an axis with its primary branches usually show fewer irregularities than the older more basal regions. Injuries of course alter the gradient for a greater or less distance

from the part concerned. Where a main axis or a branch has been bent sufficiently to crush or injure the protoplasm the susceptibility is very high unless the protoplasm is already killed, and death usually proceeds in both directions from such a point of injury, but its progress basipetally is usually the more rapid. In general the older parts of the thallus are likely to have received a greater number of such injuries than the younger and the more frequent irregularities in the gradients may be due in part to this, but there is no doubt that with the slowing down of the activity of the apical region of an axis, the gradient undergoes a leveling down and slight local differences in activity in different regions of the cell may determine irregularities in the course of death.

In a plant so delicate as *Bryopsis* it would probably be very difficult to obtain an axis with its branches which would show a perfect basipetal death gradient in all parts. Not only the greatest care in collecting and handling but also absence of injury and a fairly uniform environment for at least a considerable period before collection would be necessary conditions. The point of interest is not the appearance of local or regional irregularities, which are to be expected, but the general regularity.

There can be no doubt that the uninjured axis of *Bryopsis*, in good physiological condition, whether it is a lateral branch or a main axis, shows a basipetal susceptibility gradient, *i. e.*, a gradient in which the progress of death is basipetal and that each system of axis and primary branches as a whole shows a similar gradient.

In my material, which had remained in the laboratory for twenty-four hours before I obtained it, death was already beginning in some of the axes, undoubtedly in consequence of laboratory conditions, as no special care had been taken to keep the plant in good condition In all such cases the dead parts were readily distinguishable from the living by their failure to stain with neutral red, and it was observed that such death began apically and progressed basipetally in each axis and system of axes, *i. e.*, the susceptibility gradient was the same as in KCN. Scarcity of material made it impossible to test susceptibility to other agents and conditions but the observations and experiments on other species leave no doubt that the axial gradients in

susceptibility to KCN are simply a special case of a very general relation between axiate organisms and their environment.

Cladophora sp.

Various specimens collected at various times, first stained with neutral red, then killed in KCN *m*/50, show a basipetal gradient in staining and in death and decoloration. Apical regions stain most rapidly and most deeply and staining progresses in general basipetally. Death in KCN also begins apically and progresses basipetally. Of course exceptions to this general rule appear frequently, particularly in the older parts of the plant, where the gradient has become less distinct, and environmental factors may have affected one cell or another, or a group of cells. Nevertheless, the general basipetal course of death is apparent even to the naked eye in plants previously stained with neutral red.

As in *Enteromorpha* (Child, '16*a*) a branch is in general more susceptible than that level of the axis from which it arises. Death progresses to the base of the branch and the cell of the axis from which the branch arises usually dies considerably later.

As its ability to live under infavorable conditions would suggest, *Cladophora* is very insusceptible to KCN. In KCN *m*/50 death of the apical cells begins only after several hours and the basal regions of the main axes die only after 20–30 hours. In this respect it contrasts sharply with *Bryopsis* where death in KCN *m*/50 begins in ¼–1 hour and the whole plant is dead in 2–4 hours.

When *Cladophora* is killed in a sea-water solution of neutral red alone, death and decoloration are much less rapid than in KCN, requiring several days for completion, but the point of chief interest is that the death gradient is the reverse of that in KCN *m*/50. Death begins in the basal region, progresses acropetally in each axis and the apical cells are the last to die. This reversed gradient is like the acclimation gradient (Child, '13*a*, '13*b*, '15*c*, Chap. III.) observed in animals, where the rapidity and degree of acclimation vary directly with the rate of metabolism or physiological condition in different regions when the concentration or intensity of the external agent is not too high. In true acclimation, however, there is more or less approach to

the metabolic rate existing before the action of the external agent and it is not yet certain that such a change occurs in this case. Certain reversals of the gradient in *Griffithsia* described below (p. 430) where true acclimation is out of the question, show that reversal does not necessarily mean acclimation.

In *Enteromorpha* also, where the susceptibility gradient to high concentrations is like that of *Cladophora*, basipetal (Child, '16*a*) a reversal of the gradient often appears in neutral red, and in various other species more or less reversal has frequently been observed in neutral red. These and other cases of reversal are discussed in a later section (p. 436).

One series of observations made on portions of a single plant of *Cladophora* gave results very different from those recorded above as regards neutral red. Portions of this plant stained with neutral red showed no decoloration even after a week or ten days, although to judge from the contracted and disintegrated appearance of the protoplasm death had undoubtedly occurred. Other portions stained and then killed in alcohol 10 per cent. and 5 per cent. and in ethyl ether 4 per cent. likewise showed no decoloration during three or four days, as long as the preparations were kept, although the altered appearance of the protoplasm even after a few hours gave every indication that death had occurred. In these cases the apical regions for a length of several cells were stained an opaque black, and other portions were deep purple, the color indicating a much higher acidity within the cells than that usually observed. Portions of the same plant in KCN $m/50$ after staining with neutral red showed the usual basipetal decoloration gradient and were completely decolorized after twenty-four hours like other specimens examined.

This case occurred at the end of my stay at Woods Hole so that there was no opportunity for further tests and it is mentioned here only because of the possibility that others, attempting to repeat my experiments, might obtain such results as these. The same behavior as regards neutral red was observed once before in a single test of a fresh water species of *Cladophora*. In neutral red no decoloration occurred even after two weeks, although the plant was undoubtedly dead. Further investiga-

tion is necessary to clear up this apparently anomalous behavior which differs from that of other algæ examined as well as of other specimens of *Cladophora*. It is possible that these peculiar results are due to the neutral red rather than to the *Cladophora*, for with both the fresh-water and the marine form the neutral red used was a different preparation from that which had been used in other cases.

Fucus vesiculosus.

In this species young plants ranging in length from 12–15 mm. to 40 mm. constituted the material. Early in the course of experiments with this form it was found that the change in color and loss of the natural pigment of the plant was a more satisfactory indicator of differences in susceptibility than the decoloration after staining with neutral red and the results described below were obtained by this method.

In the earliest stages of development the plant is more or less club-shaped and circular in cross section, but in consequence of change in behavior of the apical cell the thallus soon assumes a flattened form except in the basal region, and a thickened midrib develops (Fig. 1). The plant is not, properly speaking, bilaterally symmetrical, since there is no differentiation of dorsal and ventral surface, but it is biradial, *i. e.*, there are two distinct axes of radial symmetry, one parallel, the other at right angles to the flattened surface. Since the plant grows primarily from an apical cell situated in the median apical region and since secondary growth in thickness occurs along the midrib, we might expect to find the regions of highest susceptibility apical and median and susceptibility gradients extending laterally and basally from these points, perhaps modified in the more basal regions, at least in later stages by the increased activity of secondary growth. Such a gradient appearly very clearly with various agents.

Sooner or later dichotomous branching begins (Fig. 2), each branch growing from a new apical cell which arises from the original apical cell of the axis undergoing dichotomy. Each branch then may be expected to exhibit the same sort of gradient as the original unbranched thallus and this is actually the case.

The natural color of the thallus is a dirty greenish brown or

yellowish brown. The color gradients have been determined in KCN $m/50$, alcohol 10 per cent., ethyl ether 4 per cent., and HCl $m/100$, and they are essentially similar in all, though the color changes differ somewhat with different agents. In all these agents during the first 3–4 hours there is a distinct loss of

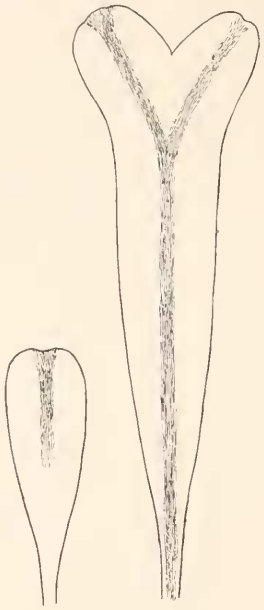

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FIG. 1. FIG. 2.

color beginning in the median apical region and progressing laterally and basipetally. This change involves the apical 5–8 mm. of the plant and does not progress further, but shades off basally into a deeper color. After this change the apical region for several millimeters is light grayish green (KCN), yellowish green (alcohol), first deep olive green soon becoming yellowish or whitish green (ether), or a dirty whitish yellow (HCl). Evidently the pigment in this region diffuses out to a considerable extent and the water is often tinged by it. This pigment loss decreases with increasing distance from the apical end, hence the color gradient from light yellowish or whitish apically to deep color approaching normal basally.

In the course of 10–18 hours in KCN, alcohol or ether the median apical region begins to show a deep brown or reddish brown color, and this color eventually progresses basipetally along the midrib, and a brownish tint spreads laterally over the thallus, the apical regions preceding, but the midrib always remains more deeply brown. In HCl this secondary change is not so clearly marked, the midrib merely becoming more yellowish than the lateral regions of the thallus.

In all cases the color changes begin in the median apical region and progress laterally and toward the base. It is impossible to determine just when death occurs at any particular level of the body in these various agents but it is probably either when the first color changes begin or when the loss of color occurs. The first change in color must mean that the reagent has penetrated the cells at least to some slight extent, and the loss of color must mean that the protoplasm or the pigment or both are so altered that the pigment is no longer held and diffuses out into the water. There can be little doubt that the cells in the regions concerned are dead when this occurs.

The point of chief interest at present, however, is the progress of the changes from the median apical region laterally and basally. This progress is more distinct in the more apical region than elsewhere, and it is in this region that the change from the embryonic to the differentiated condition is most marked. In some cases among the older thalli the cylindrical stem is apparently slightly more susceptible than the basal part of the flattened thallus, perhaps in consequence of the activity of secondary growth in thickness. The reddish brown color is probably a secondary change after death, rather than a death-change properly speaking, but its progress from the median apical region basipetally and laterally is none the less interesting, as indicating still another aspect of the axial gradients.

Callithamnion.

From the physiological point of view it seems best to describe the gradients in Rhodophyceæ with primarily monosiphonous thallus and large cells before taking up species with more complex axiate structure.

The earlier data on *Callithamnion* (Child, '16*a*) are supplemented by observations on two more species, *Callithamnion Baileyi* and a species resembling *C. Baileyi* in sympodial growth-form but with a somewhat different order of branching and strictly monosiphonous throughout, which it was impossible to identify with certainty.

In both these species the primary gradient in each unbranched axis and each cell is basipetal as tested by killing with neutral red alone, with KCN of various concentrations after neutral red and by $HgCl_2$ *m*/50,000. The basipetal gradient in single cells even along the main axes is very distinct and shows few irregularities in plants which are in good vegetative condition, provided they are not killed too rapidly.

As an axis gives rise to branches, however, the primary gradient undergoes certain modifications which are very evidently associated with the growth-form. These modifications concern the susceptibility relations of different branches along an axis. In general the farther from the apical end a branch arises the higher its susceptibility, and the rate of growth of the branches shows the same relation to the axis as the susceptibility, *i. e.*, the more basal branches grow more rapidly than the more apical. Both of these features are associated with the sympodial growth-form of these species and they are the reverse of the relations observed in the monopodial *C. roseum* (Child, '16*a*). A more extended account of these modifications of the susceptibility gradient is postponed to another time.

Griffithsia.

Griffithsia bornetiana, to which my attention was first called by Professor Osterhout, has proved to be one of the most interesting forms thus far examined, first because of the large size of the cells and the conspicuous character of the death changes, and second because the gradient very readily undergoes alterations, both in nature and under experimental conditions.

To the naked eye the color of the plant is usually rather more reddish or less brown than that of most related forms. The cells of the monosiphonous axis are readily visible to the naked eye, the longer more basal cells being often several millimeters in

length. Microscopically by transmitted light the color may be described as a brownish pink. The cells are translucent and the chromatophores and numerous nuclei are readily seen. Before death the cell surface undergoes certain changes in appearance resulting from the aggregation of minute granules or semi-fluid particles, this change differing somewhat in degree in different cells, even of the same plant, and with different agents. These aggregations are not infrequently seen in living cells under other natural or experimental depressing conditions and undoubtedly result from the activity of the living protoplasm. The occurrence of death, however, is indicated by the rapid diffusion of the pigment out of the chromatophores and into the vacuole of the cell which becomes a brilliant rose pink by transmitted light and with the loss of the pigment the greenish color of the chlorophyl becomes visible in the protoplasm. By reflected light cells which have undergone this change appear orange yellow and opaque. Diffusion of the pigment to the exterior may be very slow, but there can be no doubt that this change marks the death of the protoplasm, and it is so striking that its beginning and course can be followed without the least difficulty.

In examination of the gradient in *Griffithsia* the substances KCN *m*/50, *m*/100; ethyl alcohol, 10 per cent., 5 per cent.; ethyl ether, 3 per cent., 2 per cent., 1.5 per cent.; HgCl *m*/500,000, *m*/250,000, *m*/50,000, *m*/1,000; $CuSO_4$ *m*/50,000 approx., have been used and some observations on the axial differences in susceptibility to high temperature and confinement have been made.

Within certain limits all these agents and conditions give the same results in axes which are in good physiological condition and in the active vegetative stage. The apical cell is most susceptible, and the course of death is basipetal from cell to cell and usually within the single cell in the more apical regions. In the plants examined most of the older axes consisted of 12–20 cells and the gradient is very often perfectly regular in the first 5–8 cells from the apex downward. Below this modifications and irregularities become more frequent, though the general gradient is often very regular all the way to the base. In the basal half of such axes the cells have commonly undergone a

secondary elongation at the basal end and these cells often show a double gradient, *i. e.*, the apical and basal ends are regions of highest susceptibility and death progresses toward the middle or a region somewhat below the middle of the cell. This appearance of a secondary region of high susceptibility in the basal part of a single cell where secondary growth is occurring is paralleled in multicellular axes where secondary growth occurs in the basal region as in *Ectocarpus* (Child, '16*a*), and a similar phenomenon appears in many of the lower animals as a secondary growing region at the basal posterior end, which may give rise to new individuals (Child, '13*b*) or to segments (Hyman, '16). Sometimes the gradient in the elongated cells of the basal region is completely reversed.

The rhizoid of *Griffithsia* possesses a susceptibility gradient, the apical end, the tip of the rhizoid, being the region of highest susceptibility. In general the susceptibility of the apical end of the rhizoid is considerably lower than that of the apical cell of the vegetative axis. In these respects the rhizoid shows much the same physiological relation to other parts as does the "stolon" in the hydroid *Tubularia* (Child, '15*c*, pp. 91–92, 132–133).

In *Griffithsia*, however, the degree of individuation (Child, '15*b*, Chap. IX.) is not high, the axial gradient is not very permanently recorded in the protoplasm and therefore readily undergoes modification under altered external conditions. It is possible to eliminate or reverse the gradient experimentally in various ways, *e. g.*, by exposure to high temperature, and plants or cells which have been injured or have been living under unfavorable conditions show alterations of the primary gradient. In general in the vegetative stages the more nearly normal the physiological condition, the more distinctly and uniformly basipetal the gradient. An account of experiments along this line in which one external agent is used to alter the gradient in susceptibility to another is postponed to another time.

In addition to these alterations certain agents in certain concentrations alter the susceptibility gradient to themselves. For example in $HgCl_2$ $m/500{,}000$ the normal basipetal gradient appears. In $m/50{,}000$, however, and in higher concentrations there is more or less reversal in the apical region, *i. e.*, the apical cell

and often one, two or three cells next below it are less susceptible than any other part of the axis, and in this group of cells the gradient is usually acropetal, the apical cell being least susceptible of all. Similar results are obtained with $CuSO_4$. This partial reversal is a characteristic feature of susceptibility to concentrations above a certain limit of agents which are powerful coagulants of protoplasm such as $HgCl_2$ and $CuSO_4$. Acclimation is not concerned here, for it is the higher concentrations not the lower which produce the reversal. Apparently these agents decrease the permeability of the cells to themselves and the decrease is greatest in the most apical cells, where the protoplasm is most susceptible to alteration. This differential action of such agents is itself another demonstration of the existence of the gradient, and it is of interest to note that an external agent can reverse the axial gradient in permeability to itself. Various data indicate that other agents in sufficiently high concentration will give similar results, but the details are not yet worked out.

Age differences in the susceptibility of the apical as well as other cells are evident in *Griffithsia*. In general a small apical cell, *i. e.*, the cell which has more recently undergone division, is more susceptible than a larger apical cell, which has passed through a longer period of growth without division. Since different apical cells may be subjected to different external or internal conditions which influence their activity these comparisons often show exceptions to the general rule. The most uniform results as regards these age differences are obtained with a single main axis bearing a number of branches. In such a system the susceptibility of the apical cells usually varies inversely as the size.

A few observations on the form known as var. *tenuis* or as *Griffithsia tenuis*, with greatly elongated slender cells, gave results similar to these already described.

Cystoclonium, Agardhiella, Chondrus, Lomentaria.

In these forms each apparently simple stem or branch represents the orderly growth-activity of one or more monosiphonous axes and their branches, *i. e.*, each macroscopically simple axis is in reality a complex system of monosiphonous axes. In

Cystoclonium and *Agardhiella* each macroscopic axis consists of a single monosiphonous axis with its branches and the vegetative tip is a single cell, while in *Chondrus* and *Lomentaria* each macroscopic axis consists of a number of monosiphonous axes and their branches, and the vegetative tip consists of a group of cells, the apical cells of the main monosiphonous axes. Since these plants show very definite macroscopic axiation, it is of interest to determine whether general axial gradients exist in these axes. Such gradients correspond to the general gradient in the system of main axis and branches of *Callithamnion*. The use of neutral red is not necessary with these four genera, for the changes in color of the phycoerythrin with different killing agents and its diffusion out of the cells indicate very clearly the differences in susceptibility in different regions.

Cystoclonium purpurascens, in the few fronds examined, was found to possess a very uniform basipetal susceptibility gradient, both in the single axes and in the frond in general. Some branches of the frond are very evidently inhibited in their growth and remain short (Kurztriebe) and it is of interest to note that such branches almost always show a lower susceptibility than those which have undergone more rapid growth.

In *Agardhiella tenera* the gradient is also very uniformly basipetal in each axis for several millimeters below the apical end. In KCN $m/50$ which is of course alkaline, the color changes from the normal reddish brown to orange yellow, and this gradually changes to green as the pigment diffuses out. In HCl $m/5$ it first becomes deep purple, and this gradually fades to a dull purplish white with the loss of the pigment. In HCl a distinct basipetal decrease in cell turgor precedes slightly the first change in color, and in KCN accompanies it.

In well developed fronds the purple color begins to appear apically after 15–30 minutes in HCl $m/5$, and after two hours the whole frond has become purple and the apical regions are fading to whitish. In KCN $m/50$ the apical regions begin to turn yellow after about one hour, and after 6–7 hours the change has passed over the whole frond and the extreme apical regions show a slight greenish tint. The final change to purplish white in HCl and to green in KCN is complete only after one or two days.

In regions more than 5–10 mm. from the apical end of the axes the color change is often somewhat irregular and appears first in small areas scattered for some distance along the branch, but even in these regions the progress of death is in general basipetal. At these levels secondary growth in thickness is occurring and it may be that the areas of higher susceptibility represent the apical ends of groups of the monosiphonous axes composing the plant body, which are growing more rapidly than others about them.

In large fronds 15–25 centimeters long the middle regions of the main branches or stems for several centimeters are very commonly less susceptible than either more apical or more basal regions. That the low susceptibility of this region represents a real difference in physiological condition in fronds where it is present is clearly shown by the fact that it is thickly covered with the colorless unicellular hairs characteristic of the species while other parts of the plant show few or none of these hairs. Usually also the color is somewhat lighter than that of other parts of the plant. Undoubtedly this region of low susceptibility is of secondary origin since the younger fronds and main branches do not show it, and the fact that in the plants examined it was limited to these parts of the main branches and stems which were most thickly surrounded by other branches suggests that it may be merely a result of insufficient light or oxygen, or possibly of injurious metabolic products, in other words that it is an incidental result of the crowding of the numerous axes in this region.

The great development of hairs in these regions of low susceptibility suggest that hair development is associated with a low metabolic rate in the cells from which the hairs arise. If this suggestion is correct, the hairs appear first in this middle region because for some reason the metabolic rate is lower there than elsewhere. As the plant becomes physiologically older and its metabolic rate in other regions decreases, hairs may of course appear elsewhere. I have found that plants thickly covered with hairs usually show a lower susceptibility than those with few or no hairs.

In neutral red partial reversals of the gradient in the extreme

apical regions have been observed and in low concentrations of KCN *m*/500 the color-change begins only after 3–5 hours and appears first in the middle region of the main branches and stems, *i. e.*, the regions which are least susceptible to the higher concentrations. From these regions it progresses acropetally and basipetally the tips of the branches being usually the last parts affected. With concentrations of KCN between *m*/500 and *m*/50 or in plants which are somewhat more susceptible to *m*/500, mixed gradients may appear. The color-change may begin and progress basipetally in the apical regions and later it may begin in the middle region and progress more or less acropetally. In plants kept in the laboratory for several days the gradient shows a partial reversal, the susceptibility of the apical 3–5 millimeters of many branches being lower than that of the levels next below.

Chondrus crispus, the common "Iceland moss" with flattened dichotomously branching body, shows a very beautiful basipetal gradient in KCN *m*/50, alcohol 5 per cent., and HCl *m*/10. The first change in color from the deep red-brown or purple-brown appears in the median apical region of each ultimate branch and progresses laterally and basally in extremely regular manner. In KCN this first change is to whitish green, in alcohol to a rose-red or pink, in HCl to a fine violet or purple. Following this change in color there is gradual loss of the pigment by diffusion to the exterior, and the plant becomes whitish and finally almost pure white in KCN and alcohol and white with a trace of purple in HCl. This loss of pigment also begins in the apical region and progresses basipetally. In the concentrations mentioned above the first change in color begins in 1–4 hours and after 30–40 hours the loss of color is complete even to the base.

In plants which are in bad condition, those which have been torn loose and washed about by waves, the susceptibility is in general lower and often lowest of all in the apical regions. Where part of a frond has been torn or broken off and new axes have recently regenerated on the old basal portion the young axes show a much higher susceptibility than the old portion.

Tests of susceptibility with KCN *m*/50 and $HgCl_2$ *m*/50,000 both without and after neutral red, and with neutral red alone, made on a few plants of *Lomentaria uncinata* found detached in

shallow water after a storm showed in most axes the usual basipetal gradient, but in some cases the progress of death was irregular or even acropetal. In most species examined plants detached and washed in by the waves show reversals and irregularities much more frequently than those collected *in situ*, and in *Lomentaria* the irregularities observed are doubtless due to bad condition, but since this species was not found *in situ* there was no opportunity for checking the results.

General Discussion.

From the data recorded here and in the preceding paper it is evident that a gradient in susceptibility is a characteristic feature of the thalli of axiate forms among algæ. In the cases described in the present paper the apical region is primarily the region of highest susceptibility and the decrease is basipetal in each axis. Under unfavorable conditions and in many cases with advancing age, this primary gradient may be altered by local alterations in metabolic activity, by physiological isolation of certain regions, and in many other ways.

In some plant axes the growing tip and the region of highest susceptibility are at the attached or morphologically speaking the basal end and the susceptibility decreases toward the free "apical" end. Some cases of this sort are found in the hairs of certain algæ, *e. g.*, *Fucus* and will be considered at another time.

The significance of the axial differences in susceptibility as indicators of general metabolic rate or condition has been sufficiently discussed elsewhere (Child, '13*a*, '15*b*, Chap. III., IX., '15*c*, Chap. III.). The similarity of results with different agents shows very clearly that the general susceptibility relations depend not upon the specific chemical constitution of a particular agent, but rather upon the fact that many different agents injure and kill protoplasm and that the physiological or metabolic condition, vitality, or whatever term we prefer to use, is a factor in determining their effectiveness as killing agents.

In considering alterations of the gradient it is necessary to distinguish those which occur in low concentrations of KCN and other highly toxic agents or in slightly toxic agents such as neutral red, from those which occur in high concentrations of highly toxic agents such as $HgCl_2$ and $CuSO_4$.

There is first the possibility that the reversals in low concentrations and slightly toxic agents, *e. g.*, in *Cladophora* and *Enteromorpha* with neutral red and in *Agardhiella* in neutral red and KCN *m*/500, represent a partial acclimation. In the lower animals the capacity for acclimation varies directly with the metabolic rate or condition along the axis, so that in sufficiently low concentrations the death gradient may be reversed (Child, '13*a*, '13*b*, '15*b*, Chap. III., '16*c*). True acclimation to a depressing agent or condition consists in a greater or less degree of recovery and approach to the original metabolic condition in the presence of the agent, and in general the capacity for acclimation varies directly with the original metabolic condition. In such cases the reversal of the death gradient does not represent a reversal of the original metabolic gradient along the axis, but is due to the fact that the regions of higher metabolic rate are able to adapt themselves or acquire a tolerance to the agent more rapidly and to a greater degree and so in the long run live longer than regions of lower rate.

Whether these reversals are cases of true acclimation or merely cases in which the primary effect of the toxic agent on the region originally most susceptible alters it in such a way and to such an extent that it becomes less susceptible than other regions to further toxic action must be left for further investigation to determine. It seems probable that at least some agents in certain concentrations too high for acclimation, but not high enough to kill rapidly may actually reverse the susceptibility gradient to themselves possibly through a differential decrease in permeability or an increase in aggregation of the protoplasm or in some other way.

It may be pointed out in this connection that the reversal in *Cladophora* and *Enteromorpha* to neutral red can scarcely be the result of reversal of a permeability gradient by the action of neutral red from without for the apical regions apparently take up more neutral red than other parts, but are able to resist its action longer than other parts. In these cases the reversal must result from changes which occur after the neutral red has entered the cells. The reversal to low concentrations of KCN in *Agardhiella* is also probably not primarily a surface action, for

we should expect such action to be more marked with high rather than with low concentrations.

The more or less complete reversal of the gradient observed in *Griffithsia* with the higher concentrations of $HgCl_2$ and $CuSO_4$ is evidently not identical with the preceding cases of reversal, but is probably due to a decrease in permeability to the killing agent resulting from the action of the high concentrations on the surface of the protoplast. The fact that under such conditions a more or less complete reversal of the susceptibility gradient results means that in the most active protoplasm the permeability is decreased to a very much greater extent than in the less active cells, so that even the agent which has produced the surface change is more completely excluded from those cells where the change is greatest.

These data concerning reversal of the gradient are fragmentary because attention has been directed chiefly to the demonstration of the primary or normal gradient. Further investigation of the changes and the conditions under which they occur will undoubtedly throw more light on the problems involved.

Changes in the axial gradient may also occur in the life of the plant and may be brought about in other ways than those already described. Some of these are merely the result of local action, for example a wound may reverse the gradient, at least temporarily, in regions apical to it. Other changes are due to the action of general external factors as in the case of more or less complete reversal in *Griffithsia* by exposure to high temperature. In this case the high temperature acts like the various toxic agents in high concentration, *i. e.*, the susceptibility gradient to high temperature is basipetal.

Plants which are found detached in shallow water along the shore after storms often show more or less irregularity or reversal of the gradient, undoubtedly in consequence of depressing environmental conditions, such as exposure to high temperature, intense light or drying at low tide. It is quite unsafe to base conclusions on such plants alone. In *Griffithsia* for example, a rather sensitive form, all plants collected along shore after detachment, so far as examined, show more or less reversal in the apical regions, *i. e.*, these regions have been depressed or injured

more than others. Such highly resistant forms as *Ceramium rubrum* (Child, '16*a*), however, usually show the same gradient in detached specimens as in those collected *in situ*. In fact the frequency of irregularities and reversals in the gradient and the ease with which they can be induced experimentally constitute in some degree a measure of the sensitiveness of the species to changes in environment. Experiments on *Griffithsia* to be described later will show some of the possibilities in this direction. All of these cases of alteration or reversal of the gradient, whatever the processes and conditions involved, are of interest in the present connection since they all constitute additional evidence for the existence of a gradient and its fundamental relation to the physiological condition of the plant, and the establishment of these facts is the chief purpose of these studies of algæ.

The visible death changes in the protoplasm of cells stained with neutral red and then killed either with neutral red itself or some other agent consist, as already noted (Child, '16*a*), first, in a deepening of the red color of the dye, indicating increased acidity, followed by an aggregation of the protoplasm into separate masses which rapidly contract and become black or purple. Apparently during this stage of the process there is an increase in acidity in the cell as indicated by the change in color of the neutral red, but this is followed by a more or less rapid loss of color from the masses of coagulated protoplasm and at least often the cell-contents apparently become alkaline, if the neutral red can be trusted as an indicator.

These death changes are most striking in elongated cell bodies and are clearly seen in *Bryopsis*, various species of *Callithamnion*, the hairs of *Chondria*, *Polysiphonia*, *Griffithsia*, etc., but death may occur without such extreme physical changes in the protoplasm, as in the cells of the thallus of *Griffithsia*. It seems probable that the changes characteristic of *Bryopsis*, *Callithamnion* and of the hairs of various forms occur where the layer of protoplasm is very thin and perhaps contains a high percentage of water, while the cells with thicker or a less fluid wall die without exhibiting such extreme physical changes.

The observations on susceptibility gradients in single cells in *Bryopsis*, *Callithamnion* and *Griffithsia* show very clearly that in

a continuous mass of protoplasm very considerable local, or in this case axial, differences in physiological condition and metabolic activity may exist.

In conclusion, the essential similarity of animals and plants in respect to these axial susceptibility gradients may once more be emphasized. The physiological axis is fundamentally the same as regards susceptibility relations in both groups and undergoes very similar alterations.

Summary.

1. The thalli of all axiate algæ examined show an axial gradient in susceptibility to various agents, KCN, alcohol, ether, HCl, $HgCl_2$, $CuSO_4$, neutral red, high temperature, etc. To concentrations or intensities sufficient to kill rapidly without acclimation the apical region is most susceptible, and the susceptibility decreases basipetally in each axis. This susceptibility gradient may undergo more or less complete reversal under various conditions. Certain concentrations of certain agents may even reverse the gradient in susceptibility to themselves.

2. As in animals the susceptibility gradient is in general an indicator of the vitality, metabolic rate, or physiological condition at different levels of the axis. The gradient may be altered or more or less completely reversed by change in external conditions, by advancing age, by physiological isolation of parts, etc., and the readiness with which alterations occur in altered environment is in some degree a measure of the sensitiveness of the species.

Hull Zoölogical Laboratory,
University of Chicago,
November, 1916.

References.

Child, C. M.

'13a Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V., The Relation Between Resistance to Depressing Agents and Rate of Reaction in *Planaria dorotocephala* and its Value as a Method of Investigation. Jour. Exp. Zoöl., XIV.

'13b Studies, etc. VI., The Nature of the Axial Gradients in *Planaria* and their Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., XXXVII.

'14 The Axial Gradient in Ciliate Infusoria. Biol. Bull., XXVI.

'15a Axial Gradients in the Early Development of the Starfish. Amer. Jour. Physiol., XXXVII.

'15b Senescence and Rejuvenescence. Chicago.
'15c Individuality in Organisms. Chicago.
'16a Axial Susceptibility Gradients in Algæ. Bot. Gaz., LXII.
'16b Axial Susceptibility Gradients in the Early Development of the Sea Urchin. Biol. Bull., XXX.
'16c Experimental Control and Modification of Larval Development in the Sea Urchin in Relation to the Axial Gradients. Jour. Morphol., XXVII.

Hyman, L. H.

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochætes. Jour. Exp. Zool., XX.

STARVATION AND THE RESISTANCE OF FISHES TO LACK OF OXYGEN AND TO KCN.

MORRIS M. WELLS.

I. Introduction.

The following paper is a report on experiments that were carried on at the University of Chicago during the fall and winter of the years 1913 and 1914. The object was to determine the effect of starvation upon the rate of metabolism in fresh-water fishes. The work is still in progress, but it has seemed best to record briefly at this time some of the results thus far obtained.

II. Material and General Methods.

Practically all of the experiments reported here were performed with the rock bass (*Ambloplites rupestris* Raf.). To confirm the apparent similarity of the effects of the low oxygen and the KCN treatments, experiments with tadpoles and three or four other species of fishes were performed.

All of the animals used were collected in the streams and ponds in the vicinity of Chicago. The collections were made during the months of October, November and December. The animals were brought into the laboratory at once and with a minimum amount of handling. They were weighed immediately and those that were to be starved were placed in compartments in aquaria through which tap water coming from Lake Michigan was

flowing. No attempt was made to remove the plankton from this water and if the starving animals secured food from it the amount was far from sufficient to meet their normal needs, for the loss of weight due to starvation proceeded uniformly with the exception of two weighings (see Table I.) up to the death point. The aquaria were kept free of plant growth and no food of any kind was given the animals.

After the initial weighing the starving fishes were reweighed at gradually increasing intervals. Thus at first they were weighed every other day while later a week or ten days was allowed to elapse between weighings.

It was noted that the weight of the fishes varied slightly with the temperature of the water in which they were confined just previous to being weighed. A fish which weighed 32 grams at 5° C. weighed 32.1 grams after being placed in 12° water for 15 min. Another fish weighed 71.8 grams at 5° and 72.1 grams at the end of 15 min. in 12° water. This temperature factor was eliminated by weighing the fishes rapidly when they were taken from the aquaria for the temperature of the aquarium water changed but slightly after December 1 (varied between 4° and 8° C.).

The rock bass was selected for the experiments herein recorded because this species at the time, was easily caught by seining, in the small streams in the Chicago region; and it had been noted during several years of collecting that the individual fishes seemed to fall into natural size groups which were apparently correlated with age. Five of these groups are readily distinguishable and a sixth is sometimes taken.

The smallest fishes collected weighed from 1–1.5 grams and were evidently the fry of the previous spring. The next larger group averaged from 10–15 grams and included fishes that were probably a little over a year old. The third group weighed from 25–40 grams, the fourth from 80–100 grams and the fifth from 100–125. It is at least possible that these latter groups are made up of fishes that are in their third, fourth and fifth years respectively. Occasionally still larger individuals weighing over 130 grams were taken. Not enough of this group was taken to include it in every experiment and it is probable that fishes

of more than one year's growth are included in it. The largest specimen taken weighed 424 grams.

It should be pointed out that not all the fishes collected were easily classifiable into one or another of the above groups for some were taken whose weights placed them on the border line between two groups. This was especially true in the case of groups two and three. However most of the fishes fell readily into one of the five groups and only such fishes were used in the experiments.

III. Experimental Methods.

The resistance experiments were conducted as follows. A starved fish from the experimental aquaria was placed in a large (5-liter) wide-mouth bottle (low oxygen expt.) or in a battery jar (KCN expt.) along with a control fish of the same group. The control fish was selected so that its weight was very near the original weight of the experimental fish. In most of the low oxygen experiments a continuous stream of water flowed through the bottle. This water came from an apparatus that removed all but a trace of the oxygen.[1] The water flowed through the bottle at the rate of 300 c.c. per min.[2] The solutions of KCN were made up by diluting a standard $N/100$ stock solution. The battery jars were covered with glass plates during the experiments.

In all the experiments the control and the experimental fishes were placed in the same bottle or jar. The caudal fin of the control fish was clipped at the top and that of the experimental fish at the bottom. The two were thus easily identifiable. There was no evidence that the clipping of the fins had any effect whatsoever upon the resistance of the fishes. All the control fishes were collected just previous to the performing of the experiments.

IV. Presentation of Data.

1. *Seasonal Resistance of the Fishes.*

During several years' collecting it had been noted that in nature the resistance of fishes to detrimental factors in general,

[1] For description of apparatus see Shelford and Allee, 1913, p. 214.

[2] For complete description of methods of experimentation and recording, see Wells, '13, pp. 325–29.

is lowest in the late summer and highest in the spring. To test these observations experiments with various species of fishes were run in low oxygen water. During the winter when the experiments with starvation were being carried on the seasonal resistance curve of the rock bass was worked out rather fully, for the fall and winter months. This curve is shown in Fig. 1. The solid line represents actual experimental data and the dotted portion, conclusions drawn from field observation and some few resistance experiments performed during the time represented.

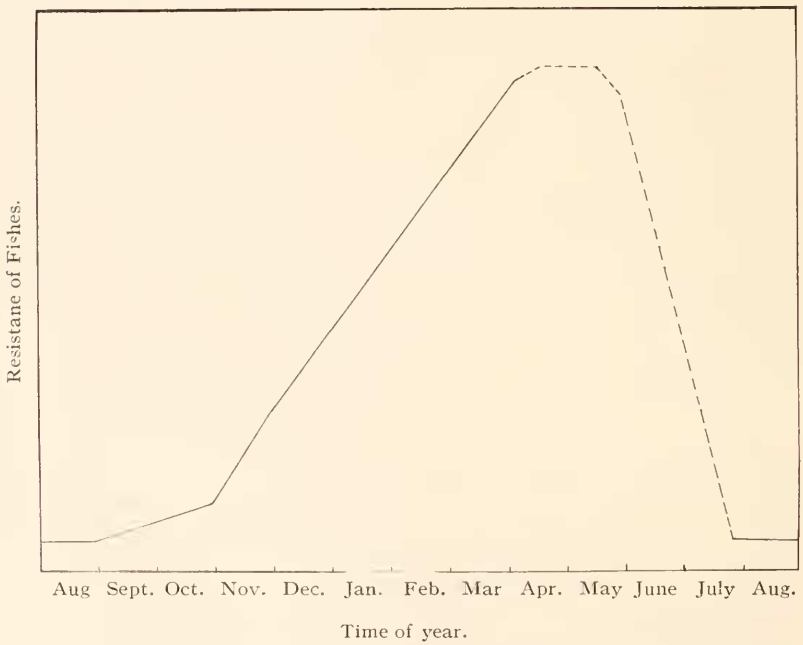

FIG. 1. Curve showing the seasonal resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The curve is based on data secured by testing fishes belonging to group 3 (*i. e.*, fishes weighing from 25–40 grams). However the relative resistance of the size groups remains practically constant throughout the seasons and the curve may be taken as representing the seasonal changes in resistance to low oxygen, of the other groups as well. That part of the curve represented by a solid line is based upon experiments performed during the months indicated. The dotted portion is based upon a knowledge of the August and March resistance, upon field observations, and upon a number of field experiments.

2. *The Process of Starvation.*

One set of six fishes was kept without food until death from starvation resulted. The following table (I.) gives the consecutive weights of the fishes during this time.

TABLE I.

Showing the regular decrease in weight of starving rock bass (*Ambloplites rupestris* Raf.). The fishes were collected on December 6 and weighed as soon as gotten to the laboratory which was two hours after capture. They were kept without food during the entire period. Numbers at head of column indicate size group to which the individual fishes belonged. Temp. 4°–8° C.

Date of Weighing.	Consecutive Weights of the Fishes in Grams.					
	1.	2.	3.	4.	5.	6.
1913						
Dec. 6	1.85	9.6	38.9	83.5	97.7	133.2
" 8	1.70	8.9	35.7	79.8	89.5	122.1
" 10	1.63	8.8	35.5	76.5	87.0	118.5
" 12	1.58	8.6	35.12	76.0	86.3	117.5
" 17	1.53	8.5	34.75	76.9[1]	85.4	117.3
" 24	1.52	8.3	34.6	75.3	84.7	117.2
" 31	Dead	8.25	34.30	74.5	83.5	116.35
1914						
Jan. 3	.....	8.1	33.9	74.15	82.1	115.9
" 10	.....	7.8	33.5	73.3	79.8	115.66
" 23	.....	7.6	32.7	72.3	77.95	114.43
Feb. 1	.....	Dead	32.45	71.9	77.6	114.1
" 5	.....	.....	32.2	71.7	76.6	113.75
" 8	.....	.....	32.0	71.0	76.3	112.7
" 15	.....	.....	31.6	70.85	76.7[1]	111.3
" 24	.....	.....	31.3	70.6	75.1	111.3
Mar. 8	.....	.....	30.5	69.1	72.5	110.0
" 22	.....	.....	29.8	66.9	69.6	102.6
Apr. 1	.....	.....	Dead			
" 9	.....	.....	.....	64.2	67.2	100.4
" 15	.....	.....	.....	61.7	64.2	96.7
" 17	.....	.....	.....	Dead	62.5	Dead
May 5	.....	.....	.....	.....	Used in Expt.	
Per cent. of weight lost	19	20	23	26	36	27

[1] Increase instead of decrease.

It will be noted from Table I. that the most rapid loss of weight comes within the first day or two when the intestine is cleared of its contents. After this the decrease is gradual, up to the death point.

The table shows two exceptions to the steady falling off in weight. Fish no. 4 lost weight steadily up to December 12, when it weighed 76 grams. On December 17 its weight had

increased to 76.9 grams. No way to account for this increase is clear. Some food substance may have gotten into the aquarium and this seems to be the most likely explanation, for one week later the weight had decreased to 75.3 grams. The fish was not dissected and the presence or absence of food ascertained as it was thought best that the series be kept unbroken. Fish no. 5 shows a similar increase in weight on February 8; again in a week the weight fell to a figure below that just previous to the increase and no other rise occurred.

3. *Resistance to Lack of Oxygen.*

Table II. is a summary of experiments performed to determine the resistance of starved fishes to lack of oxygen.

Note that Table II. shows a rapid initial increase in the resistance of the starved fishes to lack of oxygen and that this increased resistance gradually diminishes till after 53 days without food the starving fishes are considerably less resistant than the control fishes. It is also interesting to note that the decrease in resistance following the initial increase proceeds slowly for the first 39 days and then rapidly, till on the fifty-third day the starving fishes show a resistance that is not only lower than that of the control but is also lower than that of fishes of the same size but which had not gone without food for so long a time. The increase in resistance upon the part of the starving fishes is emphasized by the fact that the control fishes show a markedly increased resistance with the progress of the season as shown by the curve (Fig. 1) and the control readings in Table II.

4. *Resistance to KCN.*

When it was found that starvation results in an increase in the resistance of the starving fishes to lack of oxygen, and that this increase is followed later by a rapid and marked decrease in resistance, it was decided to test the susceptibility of fishes of the same species and in similar stages of starvation, to solutions of KCN. In this way the "susceptibility to cyanide" method that has been used so successfully with planaria by Child, was applied to fishes. A comparison of the results obtained by the two methods is interesting in that they show in general the same

relationships. Table III. is a summary of experiments performed with starving fishes in $N/25{,}000$ solutions of KCN.

Table II.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The control fishes were in every case collected just previous to the conducting of the experiment. Control and experimental fishes were placed in the same container. Water containing about .1 c.c. oxygen per liter flowed through the experimental bottle at the rate of 300 c.c. per min. Dying time is indicated in minutes. C = Control; E = Experiment.

No. Days Starved and Date of Experiment.	Serial Number and Weight of Fishes.											
	No. 1. 1–1.5 Grms.		No. 2. 10–15 Grms.		No. 3. 25–40 Grms.		No. 4. 80–95 Grms.		No. 5. 100–125 Grms.		No. 6. 130–200 Grms.	
	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.
Dec. 8.—5 days....	78	75	196	265	324	465	380	732	611	765	564	350
Nov. 25.—39 days..	80	95	180	201	300	410	327	690	540	555	555	690
Dec. 12.—53 days..	75	25	265	160	475	265	865	355	805	315	385	835

Because of the fact that a $N/25{,}000$ KCN solution is relatively more fatal than water containing practically no oxygen, the figures in Table III. are smaller than those in Table II. and the differences in the resistance of the experimental and the control fishes of correspondingly less magnitude. However Table III. shows the same initial increase in resistance (decrease in suscepti-

Table III.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to $N/25{,}000$ solution of KCN. Control collected just previous to experiment. Control and experimental fishes in same container. Dying time indicated in minutes. C = Control; E = Experiment.

Date of Experiment and No. Days Starved.	Serial Number and Weight of Fishes.											
	No. 1. 1–1.5 Grms.		No. 2. 10–15 Grms.		No. 3. 25–40 Grms.		No. 4. 80–95 Grms.		No. 5, 100–125 Grms.		No. 6. 130–200 Grms.	
	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.	C.	E.
Dec. 2.—12 days...	65	85	98	95	133	142	162	145	144	152	145	145
Dec. 2.—47 days...	84	97	92	97	128	157	145	164	148	80	140	205
Dec. 7.—52 days...	65	78	128	128	163	128	203	218	163	188	240	233

bility) upon the part of the starved fishes; also as in the low oxygen experiments, after 52 days' starvation, we note that the

starved animals are beginning to show a greater susceptibility to the detrimental condition, than is shown by the control. A comparison of the control and experimental animals at this time shows that the experimental fishes in groups 3, 5 and 6 are more susceptible, in groups 1 and 4 they are still less susceptible while control and experiment in group 2 show the same susceptibility. In experiments performed on the sixtieth day of starvation groups 1, 2 and 4 were found to be much more susceptible than the controls.

5. *Comparison of Species.*

Further evidence pointing toward a similarity in the physiological action of lack of oxygen and KCN is suggested by the results of a series of experiments that were conducted with the idea of comparing the effects of the two treatments upon other species of fishes and upon frog tadpoles. Table IV. shows the results of an experiment with KCN in *N*/25,000 concentration and a series of tadpoles and fishes whose relative resistance to low oxygen had been previously determined (Wells, '13). The relative resistance or susceptibility of the species in KCN solution is the same as had already been found for these species in low oxygen water. The tadpoles are markedly most resistant, the catfish is next, the rock bass third and the darter least. In experiments of this kind size differences were eliminated by selecting individuals of a given weight. Thus the weights varied only between 1.5 and 3 grams.

TABLE IV.

Showing the comparative resistance of different species to *N*/25,000 KCN solution The species are arranged in the order of their increasing resistance. This is the same order that they show in low oxygen water. All the individuals used weighed between 1.5 and 3 grams.

Species.	Dying Time in Hrs. and Min.
Darter (*Etheostoma cæruleum*)	35 min.
Rock bass (*Ambloplites rupestris*)	1 hr. 5 min.
Catfish (*Ameiurus melas*)	27 hrs. 50 min.
Tadpole (*Rana catesbiana*)	208 hrs. 50 min.

V. DISCUSSION.

From the data here presented it is evident that the relative deleterious effects of lack of oxygen and a *N*/25,000 solution of KCN are much the same for the animals in question. This

suggests a fundamental similarity in the manner in which these two toxic conditions interfere with the metabolism of organisms. The actual meaning of the similarity is still to be discovered.

The preceding results are of especial interest in their connection with previous work on the metabolism of the lower and the higher animals. Child ('16) has shown that flat worms (*Planaria*) that are morphologically old, if starved, can be made to retrace the metabolic steps taken toward old age and to again attain the morphological appearance and high rate of metabolism that are concomitant in nature with young worms; furthermore these worms are not apparently but *really* young and cannot be distinguished by appearance, physiological activity, or behavior from "naturally" young worms.[1] In this regressive process the planarian becomes smaller, the renewed youth being a result of the tearing down and throwing off of those morphological and physiological structures that slow up cell activity. Rejuvenation is then possible, in the planarian, because of the absence of stable structures such as are present in the higher forms.

Animals with a fixed supporting tissue may perhaps become somewhat rejuvenated by clearing the body cells of obstructions to metabolism but they cannot appreciably diminish the bulk of supporting tissue which they possess. In the mammals, starvation results in emaciation and there is no extensive reorganization such as is found in the flat worms. When a mammal is starved we get a decrease instead of an increase in the rate of metabolism, if we measure this rate by the carbon dioxide output. This depression in the rate of the oxidative processes persists throughout the entire starvation period, there being little evidence at the present time that the metabolism of a starving mammal shows any tendency to increase above the normal rate, even though the starvation period be continued till death results.

In mammals and flat worms then, we have represented, the two extremes of starvation effects so far as rate of oxidations is concerned. In man, starvation effects a depression in rate

[1] It is necessary before a starved planarian will show the same capacity for acclimation to low concentrations of killing agents such as KCN, that it be fed at least once (Child, '16, p. 164).

of metabolism which depression persists from the beginning to the end of the starvation period (Hammarsten, p. 836). In *Planaria*, starvation causes an increase in rate and this increase continues till death occurs. The effect of starvation upon the rate of metabolism in fishes is then of considerable interest for in these forms we have a group that is *structurally* midway between the flat worms and the mammals. It is not surprising therefore that fishes should possess a *physiological organization* that is apparently midway between that of the flat worms and the mammals also. In Table II., p. 447, we saw that the metabolism of starved fishes first shows a depression as in a starving mammal but that it is later accelerated as in starving planarians. The real meaning of this relation is undetermined but it is evident that the fishes resemble the higher forms in the possession of a mechanism which tends to prolong life by decreasing the rate at which the reserve tissues are used up. This is the only method possessed by mammals for withstanding starvation but fishes are also apparently capable of a certain degree of reorganization and the marked resistance which they display toward lack of food may be due to possession of both a mechanism for reserving the food stored in the tissues and to a power of rejuvenation which asserts itself when the process of starvation has, so to speak, "cleared the decks for action." At the present time, however, it is impossible to say definitely, whether or not the increase in metabolism which appears after 8 weeks' starvation, is a further insurance toward longevity or on the other hand, is a forerunner of death, being a result of the breaking down of the mechanism which has been depressing the rate of use of stored food.

That different species of fishes differ in their metabolic reaction to starvation is indicated by the results of a few experiments performed with starving bullheads (*Ameiurus melas* Raf.). With this species no stage was found where the starving individuals showed an increased resistance to low oxygen. Other experiments now under way may prove that the depression in metabolism in this species merely lasts for a shorter time than it does in the case of the more highly organized rock bass.

One further point should be considered in this discussion. It

will be remembered (Fig. 1) that the normal resistance of the rock bass rises rapidly during the fall and winter months. We are at the same time accustomed to thinking of the breeding season in most animals as being a period of high metabolic activity and there is much evidence for this belief. It is, however, at the beginning of the breeding period that we find the fishes in question showing the greatest resistance to lack of oxygen. We have then a fact that tends to contradict what has gone before, for we have been proceeding upon the basis that animals with a low rate of metabolism are more resistant to lack of oxygen than are those with a higher rate. The explanation of this phenomenon is not at present clear but it may be that the contradiction is more apparent than real. The explanation of how a fish with a high rate of metabolism can be more resistant to lack of oxygen than one with a lower rate may be found perhaps, in a qualitative rather than a quantitative investigation of metabolism. Theories of anaërobic respiration suggest that there may be present in the fish, previous to the breeding season, large amounts of certain tissues that enter readily into the securing of an oxygen supply from some source other than the free oxygen. It is hoped that something may be done toward the solution of this question in the near future.

VI. Summary.

1. It has been shown that starvation in the rock bass (*Amblo-plites rupestris* Raf.) produces first a rapid and marked increase in the resistance of the starving fishes to lack of oxygen and *N*/25,000 KCN. Later this increase disappears and after 53 days of starvation the fishes that have been without food show a considerably lower resistance to lack of oxygen and to KCN solutions than do the controls. Furthermore, the starving fishes are now less resistant than are fishes that have gone without food for a shorter period.

2. The experiments with both lack of oxygen and with KCN give results that place the fishes midway between the mammals and the flat worms so far as the effects of starvation upon rate of metabolism are concerned. In flat worms starvation initiates and maintains an increased rate of metabolism up to death;

in the fishes starvation first initiates an increased rate of metabolism which later gives way to a decrease; in mammals starvation results in a depression of metabolism which depression continues up to death.

3. The experiments recorded here tend to show that there is a fundamental similarity in the physiological disorganization caused by lack of oxygen and KCN treatments. The meaning of this similarity was not determined.

4. There is an apparent contradiction in the results in that, just previous to the breeding season, when fishes in general, possess a high rate of metabolism, the seasonal resistance curve shows a much greater resistance to lack of oxygen and to KCN than at other seasons of the year. This contradiction is yet to be explained.

VII. Acknowledgments and Bibliography.

I am indebted to Professor C. M. Child, of this department, for suggestions during the carrying on of these experiments and the preparation of the paper.

Child, C. M.

'15 Senescence and Rejuvenescence. The University of Chicago Press.

Hammarsten, Olof.

'12 A Text Book of Physiological Chemistry. John Wiley and Sons, New York.

Shelford, V. E., and Allee, W. C.

'13 The Reactions of fishes to gradients of dissolved atmospheric gases. Jour. Expt. Zool., Vol. 14, No. 2, Feb., 1913.

Wells, M. M.

'13 The resistance of fishes to different concentrations and combinations of Carbon Dioxide and Oxygen. Biol. Bull., Vol. 25, No. 6.

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University of Chicago.

TWENTY MONTHS OF STARVATION IN AMIA CALVA.[1]

W. M. SMALLWOOD.

Early in October, 1911, the department of zoölogy received for class work some forty live *Amia* from Alexander Nielson, Venice, Ohio. At the conclusion of the course, there were six live *Amia* that had not been used. These were left in the basement aquarium room in a zinc tank into which a small stream of the city water was allowed to flow continuously. The fish received no attention.

When college opened in the fall of 1911, the six fish were all alive. During a warm spell in the fall two of them died. It was thought wise to kill and fix the tissues of one of the remaining four for study. This was done. In about a month, a third one died. After this a careful watch was made to note the vigor of the remaining two. In January, 1912, one of the remaining *Amia* was killed and the tissues fixed for study. I was curious to know how long the one remaining fish would live. The individual was a female and she continued to live week after week until June 4, twenty months after being placed in the tank. At this time, the fish had become so emaciated and weak that the long tail would not stand upright and the fish swam feebly. It seemed unwise to carry on the experiment longer for fear of losing the opportunity of fixing the tissues for study. So far as the writer is aware, this is the longest period that a vertebrate has been without food while under direct observation.

The first question to be answered is the organic content of the water. Fortunately during this same period the department of chemistry[2] was making frequent analyses of this same water

[1] Contributions from the Zoölogical Laboratory of Syracuse University, C. W. Hargitt, director.

[2] The following analysis of the city water is approximately correct for the period during which you were working with *Amia calva*. Of course, the chemical composition of any water varies not only from year to year but also from month to month, so that the analysis given, while substantially correct, is not absolutely so. The results are stated in parts per million.

Solids..... 122

and a graduate student in bacteriology was making a study of the microörganisms. The latter worked in the same building and took his samples from the laboratory faucets. These two studies were carried on independently and without any reference to mine.

From the chemical analysis of the water one readily observes that the organic content is very low and that there is not enough of the organic compounds dissolved in the water to support such a large fish as *Amia*. The bacteriological study revealed the presence of several species of bacteria of which *B. coli* was the most numerous. The number of protozoa found were very few. Subsequent studies made by the city bacteriological laboratory and extending over a longer period are in general terms as follows: the bacterial content of the city water is from 20 to 40 per cubic centimeter of water when grown on agar at 37° C. After a heavy rain or a quick thaw, the bacterial content is slightly higher.

During part of the time, the water was strained through a fine piece of silk. At the end of two weeks, the silk was removed and the yellowish sediment examined. It was found to consist of diatomes.

These several independent studies show that the organic content of the water is low both in the dissolved organic content as well as in the microörganisms. The next question to be discussed is: Can *Amia* take advantage of this organic material and use any of it as food?

The several writers upon the habits of *Amia*[1] all agree that this fish is a menace to other fish, that it is savage and voracious, eating small fish and crayfish. In its natural habitat, there can

Chlorine	2
Nitrogen in nitrites	0
Nitrogen in nitrates	0.22
Ammonia free	0.04
Ammonia albuminoid	0.015

—Ernest N. Pattee, Director Department Chemistry.

[1] Bean, B., 1896, "On the Dogfish (*Amia calva*), Its Habits and Breeding," Fourth Annual Report, Comm. Fisheries, Game and Forests of New York, p. 249. Bean, B., 1903, "Fishes of New York," p. 75. Jordan and Evermann, "Fishes of North America," Vol. I., p. 113. Reighard, Jacob, 1903, "The Natural History of *Amia calva*," Mark memorial volume, p. 65.

be no question but that it lives upon the large aquatic animals. The effects of this habit is that the gill-rakers are short, blunt processes and between each there is a short space. This means that they are not effective straining organs for minute particles of food. Bacteria and diatomes easily pass between them and out through the gills into the surrounding water. The structure of the gills alone is sufficient to answer the question whether or not *Amia* used the microörganisms as food. I believe that the amount of food secured by these fish from the water is negligible.

The question which Putter,[1] Moore and others have raised in regard to the rôle that organic compounds (other than those ingested) play in nourishing animals receives a negative answer in the case of *Amia* in so far as these experiments are related to the utilization of organic compounds in solution in the water.

Color Changes.

As the breeding season approached, the green color of this female took on a brighter tint that was in sharp contrast to the usual dull color. By the middle of April, this intensifying of the color reached its height and gradually declined during the next three weeks until the regular dull shades were again assumed. On the second return of spring, a similar color change was indulged in. This was surprising as I was accustomed to think of these secondary sexual changes as following a vigorous, well-fed condition. Here the reverse is true as the animal was so emaciated as to be hardly able to swim and then in a very feeble manner. It would seem as if this secondary sexual coloration in *Amia* was a rhythmical process recurring at the period of the breeding season irrespective of bodily vigor.

Blood.

On October 8, 1913, one *Amia* just received from Alexander Nielson was etherized and the blood immediately studied. The

[1] Putter, A., 1907, "Die Ernährung der Wassertiere," *Zeit. f. allg. Physiol.*, 7, Hf. 2 and 3, pp. 283–320. 1909, "Die Ernährung der Wassertiere und der Stoffhaushalt der Gewasser," pp. 1–168, Jena, Vergl. G. Fisher. Moore, Benjamin, Edward D. Edie, Edward Whiteley, W. J. Dakin, 1912, "The Nutrition and Metabolism of Marine Animals in Relation to (*a*) Dissolved Organic Matter, and (*b*) Particulate Organic Matter of Sea-water," Biochem. Jour., vol. 6, pp. 255–297.

specific gravity of this fresh blood was 1.04. In two counts of the red corpuscles, the number was 1,680,000 and 1,600,000; while the white corpuscles were 800,000 and 400,000 in the two counts made. Some of this fresh blood was placed in .5 per cent. osmic acid for later study.

Professor Brewer's[1] chemical analysis of samples of this normal blood gave the total nitrogen in 100 grams as 69 per cent. and the total urea-nitrogen 39.5 per cent. This makes the urea-nitrogen 57.2 per cent. of the total nitrogen in the blood. The remaining nitrogen is in the form of amino-acids.

When the *Amia* that had been starved twenty months was killed, a similar study was made, giving the following results: Specific gravity of the starved blood 1.03. Red corpuscle count 400,000. There was no evidence of white corpuscles in the several counts made nor in the preparations stained with Wright's blood stain. Some of this blood was placed in .5 per cent. osmic acid.

The total nitrogen in 100 grams of this starved blood was 30.5 per cent. and the urea-nitrogen 18 per cent. which gives the urea-nitrogen as 59 per cent. of the total nitrogen in the starved blood.

At the same time that the blood of the normal and of the starved animal was being examined as just indicated, a number of cover glass preparations were made and stained with Wright's stain. These and the osmic fixed corpuscles were subsequently studied with the oil immersion lens. It was soon evident that there was no constant difference between the red corpuscles of the normal and starved animals. But to be more certain, microphotographs were made and the negatives projected onto a screen. In this manner each corpuscle became so large that it was readily measured with a millimeter scale. While these measurements were being made, the negatives of the normal and starved blood were in such order that the one making the measurements did not know whether the blood was normal or starved. When these results were checked up, it was found that the size of the red corpuscles had remained fairly constant. No evidence of any definite variation in the red corpuscles of the

[1] The chemical analyses embodied in this paper were made by Professor R. K. Brewer, M.D., of the Department of Chemistry, Syracuse University.

starved blood was noted. The corpuscles in the blood of the normal fish tended to vary slightly more than those from the starved animal.

Muscles.

The muscles of a normal *Amia* are compact coarse fibers separated by strong connective tissue into myomeres. This muscle layer is from a half to three quarters of an inch in thickness in the dorsal region. When the skin of the starved *Amia* was removed all of the firmness and compactness of the normal muscle was lacking; this was especially true in the apparent disappearance of the myomeres. The muscles in the region of the gills and operculum were similar to the muscles in a normal animal.

When the blade of a scalpel was lightly scraped over the broken down muscles, a murky, structureless substance was secured that flowed from the scalpel in drops when the scalpel was held suspended. A considerable quantity of this semi-fluid muscle tissue was fixed in osmium-bichromate, zenkers, formalin, and chrome-sublimate. One chance preparation was made just as one makes a cover glass preparation of blood and stained with Wright's blood stain. This was fortunate as it was the only one of the preparations to yield satisfactory results for microscopic study.

In preparing for the chemical analysis of this muscle, it was necessary to take all of this semi-fluid muscle tissue in the entire animal in order to secure 3 grams of dry substance.

The following data enables one to compare the composition of the muscle of the normal and starved animals. No fat was found in the starved muscles. For the significance of the following analysis, the reader should consult the numerous papers of Folin[1] and Dennis, and Van Slyke.

[1] Folin and Denis, "Protein Metabolism from the Standpoint of Blood and Tissue Analysis," Seven papers in the *Jour. Biochemistry* as follows: Vol. XI., no. 1, 1912, no. 2, 1912, Vol. XII., no. 1, 1912, no. 2, 1912, no. 2, 1912, Vol. XIV., no. 1, 1913, Vol. XVII, No. 4, 1914. "Metabolism Studies on Cold Blooded Animals," Vol. XIII., no. 2, 1912. "Note on the Tolerance Shown by Elasmobranch Fish Toward Certain Nephrotoxic Agents," Vol. XVI., no. 3, 1913. J. Bio. Chem. Vol. X. P. 15.

The total nitrogen in 3 grams of starved dry muscle was .4474.

	Per Cent.
Ammonia-nitrogen	3.1
Melanin-nitrogen	1.8
Amino-nitrogen	63.9
Non-amino-nitrogen	4.9
Nitrogen of bases	26.9
	100.6

The proportions in the normal muscle are as follows: Total nitrogen in 3 grams of dry muscle .3403.

	Per Cent.
Ammonia-nitrogen	7.2
Melanin-nitrogen	1.9
Amino-nitrogen	55.6
Non-amino nitrogen	9.4
Nitrogen[1] of bases	26.0
	100.1

A comparison of this analysis with that of the starved muscle reveals the interesting fact that the general relation of the several nitrogen compounds found in the muscle is not materially changed. A chemical analysis, therefore, does not help us in explaining the sequence of events which results in the breaking down of the muscle cell.

The histological study of the muscle shows the order in which the parts of the cells disappear, although no satisfactory preparations were obtained from material fixed in the several solutions mentioned above. The semi-fluid of starved muscle stained with Wright's blood stain did however give a fine differentiation of the muscle fibers and their cross striæ.

It has been known for some time that the sarcoplasm was broken down in extreme starvation, but I am not familiar with any observations that determine the order in which the change occurs. The untouched microphotographs, Fig. 1, shows that the cross markings in the sarcoplasm are the first structures to undergo any change. These become faint and less compact at the end of the muscle cell while toward the middle of this same cell they are unchanged. This is clearly indicated in the above figure where a normal fiber and one that is breaking down lie side by side. The muscle nuclei of each fiber are of equal size and staining reaction.

[1] This is the nitrogen precipitated by phosphotungstic acid.

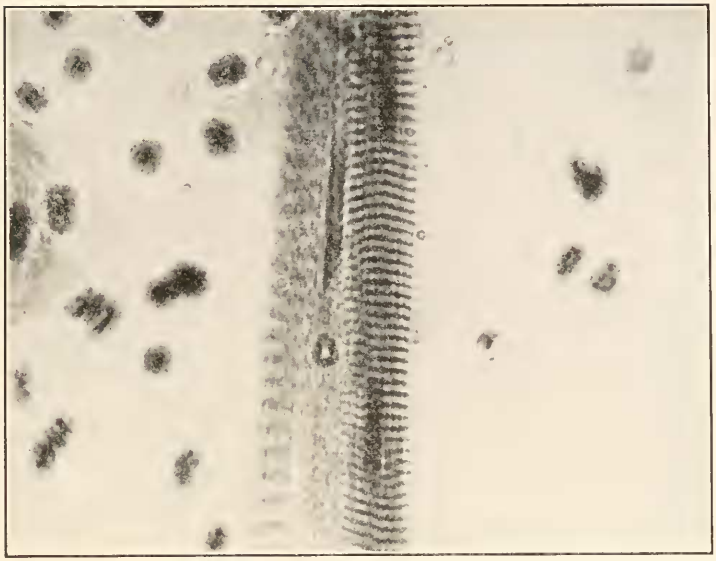

FIG. 1. Microphotograph of body muscle of *Amia calva*, showing normal and partly broken down muscle cells. Stained with Wright's blood stain. Magnification 600 ×.

In Fig. 2, a second microphotograph, are shown some normal muscle fibers, others partly broken down and one entirely empty. In the empty fiber, the muscle nuclei are still arranged along the cell wall of the muscle. One of these nuclei has divided. Three red blood corpuscles appear near this divided nucleus and furnish a good comparison. The appearance of the blood corpuscles as photographed indicates that the cells are well fixed.

These two microphotographs clearly indicate that the striæ, then the sarcoplasm and finally the nuclei is the order in which the several parts of the muscle cell break down in *Amia* during starvation.

Fig. 3 is a microphotograph of a dividing muscle nucleus and the method is certainly amitotical. These nuclei become separated from the cell wall and gradually fragment. Several smaller pieces are seen in this figure.

A variety of stains was tried on the material fixed and sectioned but the results were unsatisfactory. The muscle sarcoplasm and nuclei stained very faintly. In one slide stained with

Conklin's picro-hæmatoxylin many small cells were stained. Each of these has a distinct but small amount of cytoplasm with a definite nucleus in which the chromatin was delicately distributed. These nuclei had a decidedly healthy appearance. After trying a number of stains, I am inclined to interpret them as connective tissue cells. But I am at a loss to understand why

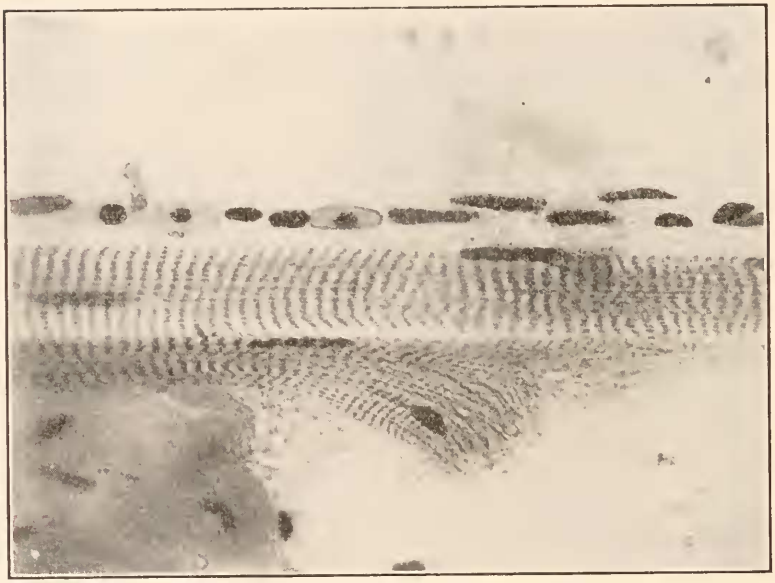

FIG. 2. Microphotograph of body muscle of *Amia calva*, showing a normal partly broken down and an empty muscle cell. Note that the nuclei of the empty cell are still arranged along the cell wall. One has divided. Magnification 600 ×.

they should be apparently so normal when all of the parts of the muscle cell stained so faintly. From their appearance one might suspect that they were associated with the breaking down of the muscle cells. I have not been able to locate in the literature any evidence that the internal secretions that are believed to be responsible for this breaking down of muscle are the product of any definite cells. It may be that part at least of the secret is discovered in these active connective tissue cells.

NERVOUS.

During this prolonged enforced fasting, the operculum was constantly raised and lowered in a regular manner. This simple

movement associated with the passage of water over the gills is correlated with the drawing in of the water into the mouth so that not only the vagus group of nerves but the trigeminal complex also is involved in this apparently simple reflex.[1] In attempting to determine what group of cells was constantly at work in this respiratory movement, one is unable to be certain which group is doing the work. There does not seem to be any

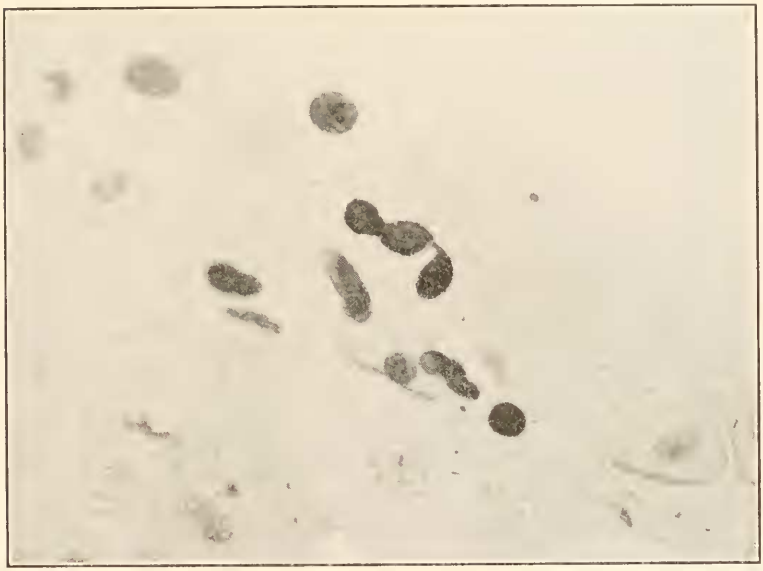

FIG. 3. Microphotograph of amitotically dividing muscle nucleus. The smaller black bodies are muscle nuclei undergoing degeneration. The red blood corpuscles serve as a measure of the amount of change that some of them have undergone.

way of determining which group of cells in the reflex chain is expending the greater amount of energy; is it the group of cells that receives the initiating stimulus or the one that sends the motor stimulus to the muscles of the gills and operculum? Several of the nerve centers associated with the trigeminal and vagus were studied in an attempt to determine the influence of

[1] Allis, E. P., 1897, "The Cranial Muscles and Cranial and First Spinal Nerves in *Amia calva*," *Jour. Morph.*, Vol. XII., no. 3. Herrick, C. Judson, 1899, "Cranial and First Spinal Nerves of *Menidia*, a Contribution upon the Nerve Components of the Bony Fishes," *Jour. Neur.*, Vol. IX.

this continued respiratory activity in the gill region but the results were not satisfactory.

The tank in which these fish were kept was in a shady part of the room and for weeks at a time the fish were not disturbed. It would seem as if the sensory components of these two nerves were as free from stimuli as it is possible to have a living animal in its normal environment. Under such conditions, the cell

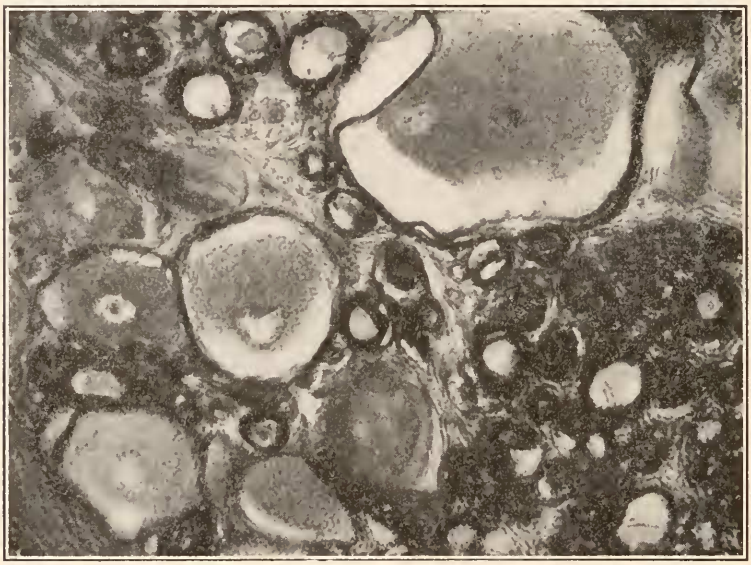

FIG. 4. Microphotograph of nerve cells in tenth ganglion. Magnification 258 ×.

bodies in the tenth ganglion would certainly not be in a state of fatigue. Rather the reverse should be the condition, *i. e.*, a condition of rest. The nerve cells which immediately govern the contraction of the muscles of the gills are located near the floor and at one side of the medulla. In selecting these as the motor nerves of the vagus for the gills, I am accepting Herrick's conclusions as already cited. If these are the correct cells to select for this study, then we should expect them to be in just the opposite condition to the sensory cells in the vagus ganglion because they have been continuously transmitting motor stimuli.

The microphotographs, Figs. 4 and 5, clearly indicate that the

nuclei are round and that the chromatin granules are uniformly scattered. A conspicuous nucleolus is present in nearly every one. The size and general appearance of these nuclei lead me to conclude that the condition of the cytoplasm is also normally fixed. The presence of large clear areas filled with sap is what is found when the living starved nerve cell is studied. It is interesting to note that both of these cells were able to do their normal

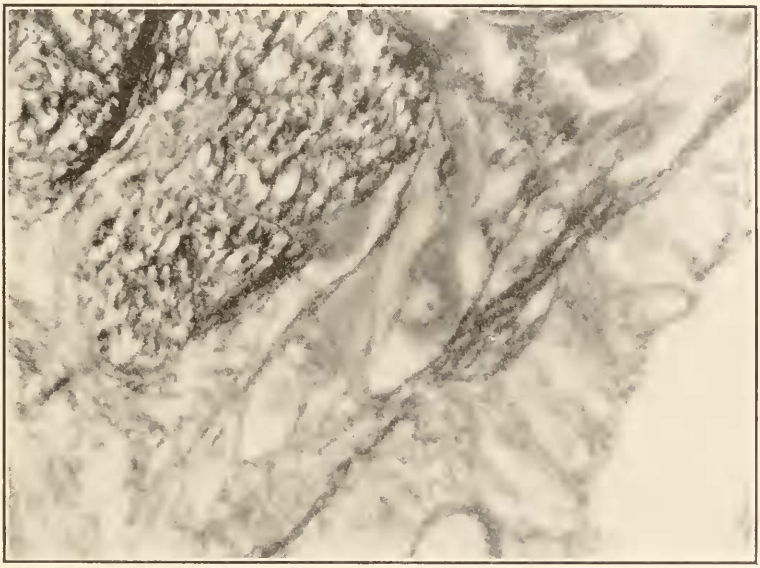

FIG. 5. Microphotograph of motor nerve cells of the tenth nerve. Magnification 258 ×.

work although in an apparent state of almost complete inanition. Many of the cells showed only about one-half of the normal amount of cytoplasm. It is also evident from these two microphotographs that there is no constant morphological difference between the sensory cells that had had a long rest and the motor cells that had been constantly working. In fact so far as I can determine there is no constant structural difference between any of the nerve centers associated with the respiratory reflexes. The fish utilized nearly its entire body muscles in order to supply food energy to the nervous system. This energy while not entirely adequate appears to have been generally distributed

in and utilized by the nervous system irrespective of the amount of work to be performed.[1]

Summary.

1. *Amia calva* is able to live at least for twenty months in an aquarium tank without food. During this time the body was furnished with food energy that was derived from the body muscles.

2. The blood does not show any definite chemical variation during this period of fasting nor do the individual red blood corpuscles undergo a definite change. There seems to be a marked reduction in the number of red and white corpuscles.

3. In the breaking down of the muscle cell, the parts of the cell disappear in the following order: the muscle striæ, then the sarcoplasm and finally the nucleus.

4. The cells of the nervous system continued to function although highly vacuolated. There does not appear to be any constant morphological change in the nerve cells that worked and the cells that rested during this long period of fasting.

5. The bright colors of the reproductive period were assumed by this starved *Amia* twice while undergoing enforced fasting.

[1] A detailed study of the digestive glands and digestive tract is being made by W. H. Kortright and will be reported at a later date.